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ENOLOGICAL INVESTIGATIONS

I. INTRODUCTION.

Special investigations in viticulture and enology have been pursued by the Viticultural Division of the College of Agriculture in fulfillment of the requirements of an act passed by the legislature of the State of California in 1909. Details of the purposes of this act are given in the introduction to Bulletin 213. In accordance with the provisions of this act the following publications have been issued:

1. Grape Growing in the Imperial Valley. In Bulletin 210, January, 1911.
2. The Principles of Wine-making. Bulletin 213, May, 1911.
3. The Extermination of Morning-Glory. Circular 69, August, 1911.
4. Hot-room Callusing. Circular 76, August, 1911.

The legislature of 1911, confirming the act of 1909, continued the appropriation to carry out its provisions. Two publications, including the present bulletin, have been issued as the result.

5. Grape Vinegar. Bulletin 227, January, 1912.
6. Enological Investigations. Bulletin 230, June, 1912.

The principal improvements in the art of wine-making contributed by the last twenty-five years are the accurate and intelligent use of sulfurous acid, the application of pure yeast to the fermentation of grapes and the control of the temperature in every stage of the process.

The proper use of sulfurous acid has made it possible to avoid completely the production of spoiled wines. No wine-maker who understands this use and applies his knowledge has any vinegar-sour, lactic, mousey, slimy, or otherwise diseased wines in his cellar. High volatile acid, unfermented sugar, persistent cloudiness, defects due in nearly all cases to bacterial growth can be completely eliminated. It enables him to have every gallon of his wine sound, dry and clear within three months or less from the crushing of the grapes. This is, therefore, the first and most important improvement in his art that the wine-maker should study.

The application of pure yeast is perhaps the next. It is the complement of the use of sulfurous acid. It may not in every case enable him to improve his best wine, but it will give much greater certainty to his operations and undoubtedly improve the average quality and increase the uniformity of his product. It prevents those strange varia-

tions of quality between casks of wine made from the same grapes by the same methods which have so long puzzled wine-makers.

The control of temperature is the final improvement which gives him absolute control over every detail of the wine-making process. In addition to the two other means, it enables him to reduce his operations to an exact method and to eliminate chance. By use of all three, he can lay out his work with a precision and certainty unknown to the old forms of wine-making. By restricting the rise of temperature during the violent fermentation and maintaining it during the after fermentation the delicacy and the finer qualities of the wine can be greatly improved. Refrigerating the wine to a suitable degree after the elimination of the sugar promotes its rapid clarification by the precipitation and deposition of salts, micro-organisms and the other solid matters which often form a persistent cloudiness in defectively handled wines. A proper rise of temperature after the wine has been cleared by the first or second racking, hastens and facilitates the maturing of the wine. The time and degree of this rise are determined by the degree of aging desired. When this is obtained, a lowering of the temperature makes it possible to keep the wine without depreciation until it can be brought by subsequent finings, rackings and other operations into the condition required by the consumer.

The general principles and practice of these improvements have been discussed in Bulletin 167, "The Manufacture of Dry Wines in Hot Countries"; Bulletin 213, "The Principles of Wine-making"; Circular 22, "Defecation of Must for White Wine"; and Circular 23, "Pure Yeast in Wineries." Experiment work on their applicability to Californian conditions has been reported in Bulletin 174, "A New Wine-cooling Machine," and Bulletin 177, "A New Method of Making Dry Red Wine."

Cooling devices have been installed in a large number of Californian wineries and their utility is very generally recognized by our wine-makers. Pure yeast has been used with success for several years in a limited number of wineries in the State and would become more general if its merits and ease of application were better understood. Sulfurous acid has rapidly come into very general use, but many wine-makers have an imperfect understanding of its effects and of the best methods of use.

The present bulletin is an attempt to popularize the last two improvements and to clear up some uncertainties regarding their use.

Investigations were made during the last season to determine to what degree the injurious micro-organisms could be eliminated and the wine yeast encouraged by the use of sulfurous acid and pure yeast and to discover the best methods and doses in various cases. Some of

these investigations were conducted in the laboratory and some at the winery of Mr. J. E. Colton, who very obligingly offered us the necessary facilities. Some tests were made on a scale large enough to demonstrate their applicability to industrial conditions in California, and to determine how much improvement could be expected from the use of sulfurous acid and pure yeast, which are easily and cheaply applied without the asistance of cooling devices which require more trouble in applying and more expense in installing.

II. SULFUROUS ACID IN WINE-MAKING.

A. General Considerations.

(a) *Historical.*—The use of sulfurous acid in wine-making, dating from remote antiquity has within recent times undergone remarkable developments.

The Romans used sulfur to mix with the pitch, wax, incense and spices which they steeped in their wine or burnt in the vessels in which they stored it. The literature of more modern wine-making contains numerous favorable references to the utility of the fumes of burning sulfur for the treatment of casks in which wine was to be kept. Many authors recommended the addition to the sulfur of orris root, ginger and similar aromatic substances. Such additions were doubtless a survival of the ancient practices and show that wine-makers were still ignorant of the true nature of the action of sulfur fumes.

(b) *Modern Developments.*—Only within the last twenty-five years have we begun to formulate a correct theory of the effects, beneficial and injurious, of "sulfuring," enabling us to make use of the former while avoiding the latter. The development of this theory we owe to the general progress of applied science and especially to the researches of Nessler, Weigert, Strucchi, Bouffard, Martinand, Laborde, Wiley and many others who have investigated the practice from the chemical, industrial, and hygienic sides.

At present, sulfurous acid is used in some form and for some purposes in every winery and every wine cellar where good wine is made and where anything but the most primitive and elementary notions of the art of wine-making exist.

(c) *Opinions of Experts.*—Babo and Mach, in the last edition (1910) of "Kellerwirtschaft," the principal work on wine-making in the German language, say: "Sulfuring is indispensable." "In cellars where the practice is unknown, it is hard to find completely sound or merchantable wines." J. Weinmann, in the last edition of "Manuel du Travail des Vins Mousseux," recommends the use of 3 to 5 grams

of bisulfite of potash per hectoliter of must in the manufacture of champagne. L. Mathieu, speaking for Burgundy, says (1911) "Wine of the highest quality is obtained by the previous sulfuring of the must." J. Laborde, discussing the preparation of the white wines of the Gironde, states, (1910), "The use of sulfurous acid in white wine is a benefit—even a necessity." E. Dupont, giving the results of his observations on the use of sulfurous acid in the south of France, says (1910), "The practice should become a part of the general method of wine-making," and further, "We are obliged to recognize that since the use of sulfites in the fermenting vats has become general, the number of badly made, defective or diseased wines, formerly so frequent, has diminished considerably; in fact, such wines have almost disappeared from commerce." Finally, at the International Congress of Madrid (1911) five resolutions were adopted having reference to the improvement of wine and its methods of manufacture. Three of these resolutions speak in favorable terms of the use of sulfurous acid and the other two recommend methods which are based on its use.

Among modern authorities there are but few dissenting opinions, and these come from chemists or medical men who have little or no knowledge of wine, and whose views are based on observations of the action of sulfurous acid in cases where the objects and effects are totally different from those of its proper use in wine-making.

It may be taken as demonstrated, therefore, that to obtain the best wine from the point of view both of the producer and of the consumer, the rational use of sulfurous acid is necessary. No substitute has yet been found of which the efficiency has been demonstrated. There are serious objections to all which have been proposed and none of them accomplish all the desirable objects attained by the use of sulfurous acid.

(d) *Legal Limitations.*—In order to obtain these results, however, it is necessary that the wine-maker should understand clearly how the sulfurous acid acts and the limits within which it must be used. A lack of this understanding on the part of the wine-maker and a confusion of the effects of the free acid with those of the combined acid, as it occurs in wine, on the part of earlier investigators has led to legal limitations on its use. These limitations were at first so narrow as practically to prohibit its use, but with increasing knowledge of the facts, they have been gradually widened until they are now sufficient to cover all the amounts which the intelligent wine-maker needs. The present tendency of pure food laws seems to be to place the limitations at about .035 per cent or 350 milligrams per liter of total sulfurous acid of which not more than 70 milligrams may be free. There is no need ever to exceed these limits, and the wine-maker who does so will in

most cases injure the quality and merchantable value of his wine irrespective of the legal limitations. For the protection of the consumer there is little need of legal limitations, as an excess which approaches an amount harmful to the consumer will so depreciate the selling value of the wine that the abuse would tend to cure itself. The establishment of legal limits, however, has been of great use to the wine-maker by making it necessary for him to study carefully the methods of applying sulfurous acid, and of graduating the amount to the case in hand in order to obtain the maximum beneficial effects.

B. Properties, Preparation, Use and Effects of Sulfurous Acid.

(a) *Properties.*—Sulfurous acid (SO_2) is a colorless gas 2.2 times as heavy as air, easily recognized by its characteristic odor. Sulfur itself has no odor and the so-called "sulfur smell" is due to the SO_2 produced when sulfur is burned.

The gas is soluble in water in the proportion of 30 to 45 volumes at ordinary temperatures. At the temperature of 20° C. one volume of water will dissolve 36.4 volumes, or one pound of water will dissolve .104 pounds of the gas. The gas liquefies at ordinary atmospheric pressure when its temperature is lowered to —10° C. At the temperature of 20° C. it exerts a pressure of 3.25 atmospheres or 40.6 pounds per square inch.

Sulfurous acid gas will not burn like hydrogen or illuminating gas nor support combustion like air or oxygen.

It has a bleaching action on organic colors which is sometimes utilized in decolorizing fabrics, nuts, dried fruits, etc. It owes this property to its power of forming colorless compounds by combining with the coloring matters. These compounds can usually be broken up by oxidation and the color restored. Pink must or wine can be made white by treatment with SO_2, but on aeration the color returns. The bleaching action thus differs materially in character from that of chlorine which destroys the color, or that of charcoal which removes it.

When dry it is inactive. In the disinfection of rooms, the air is first saturated with moisture by means of steam which allows the SO_2 to exert its germicidal properties.

Compounds are readily formed by SO_2 with certain organic substances called aldehydes of which formaldehyde and acetaldehyde are typical. The latter occurs in wine together with others similar in very small quantities. These compounds are what are known as the "combined" form of SO_2 as it occurs in wine. This property has been utilized for the removal of SO_2 from wine by the addition of urotropine. This substance acts by breaking up slowly with the evolution of for-

maldehyde. As the latter is harmful, the process is useless and illegal when applied to wine.

Similar combinations take place between SO_2 and the sugar of grape must. These combinations are fairly stable and they seem to constitute the main part of the combined SO_2 in grape must.

When introduced into wine or grape must, some of the SO_2 fails to enter into any of these combinations. It is this part which is called the "free" SO_2. It is this form which has the active antiseptic and germicidal properties utilized in wine-making. It is also this form which in excess is harmful to animals and men who consume excessively sulfured products, and to plants growing in the vicinity of factories which give off "sulfur fumes."

In solution in water, it readily attacks alkalies, metallic oxides and most metals, forming salts termed sulfites, bisulfites, and metabisulfites, according to the proportion of SO_2 which they contain. Dissolved in water, therefore, it is a true acid. Its acid properties are weak, that it to say, it is readily liberated from its salts by other acids such as sulfuric, tartaric, citric, and acetic. This explains the odor of burning sulfur given off by a fermenting vat when the grapes have been treated with metabisulfite. The SO_2 is set free by the tartaric acid of the must.

(b) *Preparation and Use in Wineries*: 1. SULFUR FUMES.—When sulfur is burned in the air, oxygen is taken up and sulfurous acid gas is formed. The chemical reaction involved is expresed thus:

$$S + 2O = SO_2.$$

Sulfur + 2 Oxygen = Sulfurous acid.

This is the reaction which takes place when a cask is disinfected by burning a piece of sulfur tape. Sulfur wicks or matches are made by coating strips of asbestos or of thin linen or cotton cloth or of tough paper with sulfur. The fabric is cut into strips about $1\frac{1}{4}$ inches wide and 10 or 12 inches long. These strips are then dipped into melted sulfur kept at the lowest temperature at which it will remain liquid. By dipping two or three times and allowing the sulfur to harden between times, a thick coating of sulfur can be obtained. As the burning of the cloth core produces ill-smelling fumes the smaller the proportion of cloth to sulfur the better.

When used for sulfuring small casks, these wicks are usually suspended by means of a sulfur bung and burnt in the cask. A sulfur bung consists of a wooden bung sufficiently long and tapering to fit all sizes of bung holes, and furnished with a piece of iron wire 10 inches to 15 inches long inserted in the bottom of the bung at one end and turned up to form a sharp hook at the other.

There are several serious objections to using sulfur in this way. Part of the sulfur melts and falls to the bottom. If it burns there, it injures the staves of the cask, if it fails to burn it may communicate a bad taste to the wine. Part volatilizes and is deposited as fine flowers of sulfur on the walls of the cask where it may later be changed into hydrogen sulfide by the action of yeast. Hydrogen sulfide is the cause of the "rotten egg" smell of some wines which is the same as that familiar at sulfur springs. Another portion of the sulfur forms evil-smelling compounds with the cloth core of the wick and still another portion is oxidized to *sulfuric* acid, SO_3.

To prevent the falling of the molten sulfur to the bottom of the cask the sulfur wick may be burned in a "sulfur cage," consisting of a narrow metal or earthenware cylinder with perforations on the sides

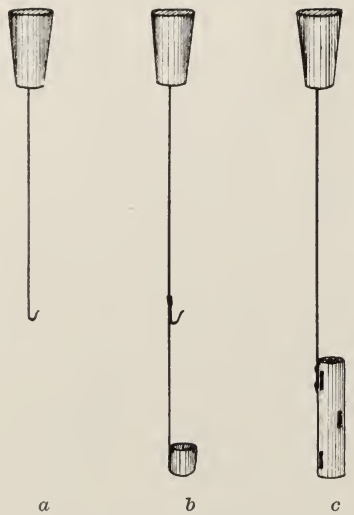


FIG. 1.—Devices for burning sulfur in casks.
a, sulfur hook; *b*, sulfur cup; *c*, sulfur cage.

to admit air. This cage has a solid bottom to catch the melting sulfur and is suspended in the cask by means of a sulfur bung. Sulfur cups of similar design are made in which the sulfur can be burnt directly and the defects due to burning cloth obviated. The latter are not very convenient to use, however, except where very small quantities of sulfur are needed. In sulfuring large casks furnished with a manhole at the bottom, the sulfur is usually burned in an iron or earthenware pan on the bottom of the cask. This pan should be supported by a brick or other means of protecting the staves from the heat of the burning sulfur.

Various devices for sulfuring are in use in which the sulfur is burned outside of the cask. One of these consists of a barrel from which one

head has been removed. It is inverted over a pan in which the sulfur is burned. Air is admitted through holes near the ground and the sulfur fumes introduced into the must or wine by means of an air pump connected with the top of the barrel and the cask to be sulfured. The sulfur fumes quickly corrode a metal pump. See Fig. 2.

A better type of sulfuring machine and one in more common use is illustrated in Fig. 3. It consists of a stove in which the sulfur is burned connected with a tall cylindrical vessel in which the sulfur fumes are brought into intimate contact with the must. The heated gas and air entering the bottom of the cylinder pass over the surface of the must, which flows over a series of inclined plates and the whole

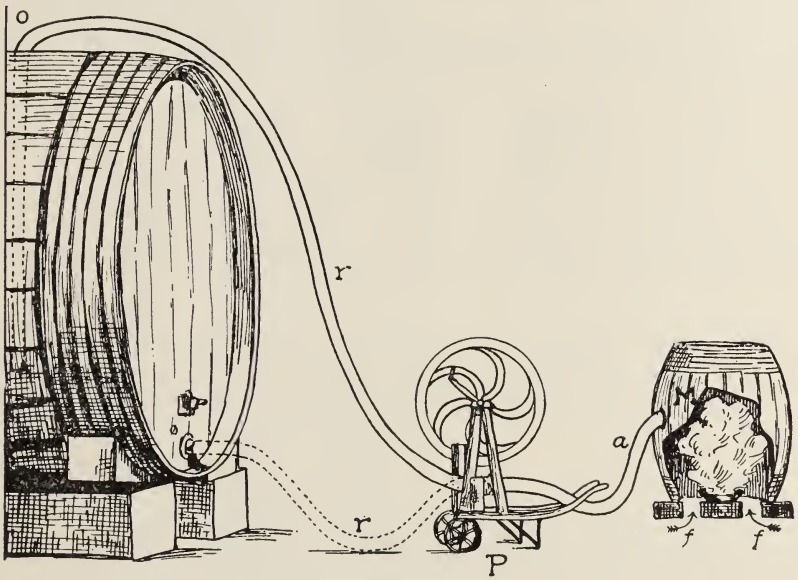


FIG. 2.—Method of sulfuring must with a pump.

M, small cask with one head removed, serving as a chamber in which to burn the sulfur; *f, f,* openings for entrance of air; *a, r,* hose through which the sulfur fumes are forced into the cask of wine by means of the pump, P.

of the SO_2 produced is absorbed. By interposing a water trap between the stove and the cylinder, and forcing the fumes through this trap by means of an air blast, the volatilized sulfur and sulfuric acid may be removed.

This device removes most of the objections cited above, but the extreme uncertainty of measuring and the difficulty of regulating the amount of SO_2 introduced into the must still exist.

A device for partially removing these objections consists of a turbine turned by the must which flows over it. This turbine operates a fan which supplies the air to the sulfur stove. As the rapidity of the turbine varies with the rate of flow of the must, and the SO_2 produced

varies with the amount of air passing through the stove, a means of regulating the amount of SO_2 in accordance with the volume of must is afforded.

With even the most perfect of these devices, one other serious defect of the method of obtaining SO_2 directly from burning sulfur remains. This is the extreme uncertainty of the amount of SO_2 produced by burning a given weight of sulfur.

According to the formula of the reaction, one part by weight of sulfur

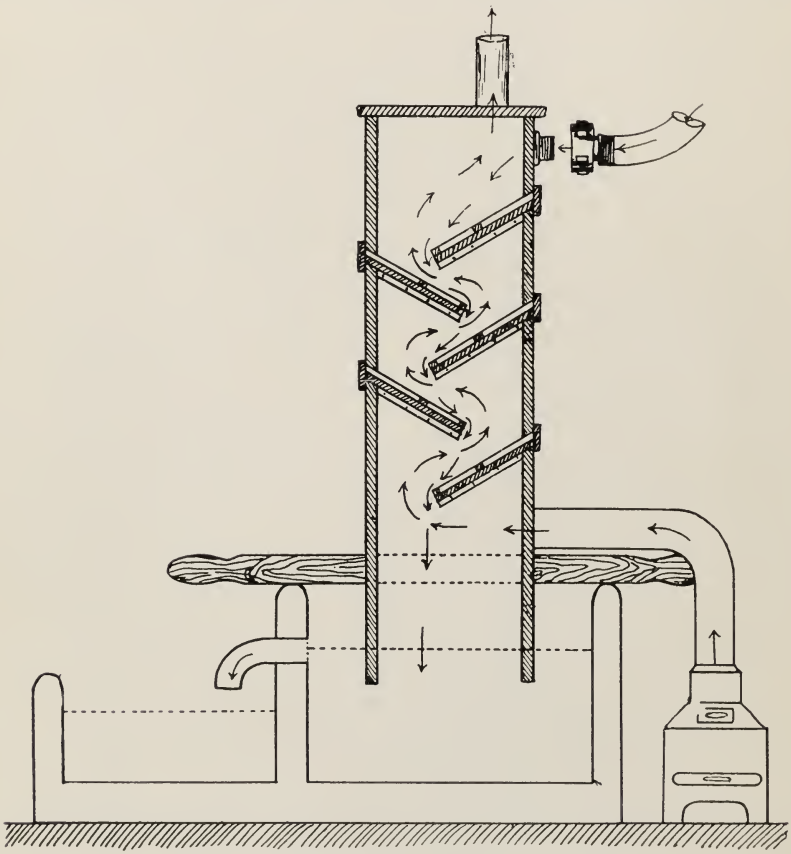


Fig. 3.—Sulfur machine.

on burning should yield two parts by weight of SO_2, or one ounce should yield .782 cubic feet of the gas at 60° F.

In practice the yield is much less than this. Pacottet states that the quantity of SO_2 actually formed when sulfur is burned in a small cask varies from 15 per cent to 60 per cent of that corresponding to the amount of sulfur consumed. This loss is due to the sulfur which is volatilized or melted without burning and to that which forms sulfuric acid. It varies with the amount of sulfur burnt in a given space

and with the mechanical condition of the sulfur. When there is a full supply of air and when the sulfur is well exposed to it, the combustion is more perfect. As the air becomes exhausted of its oxygen, combustion becomes less perfect. When all the sulfur possible is burned in a closed space, a greater proportion is lost than if a smaller quantity is burned.

When only a very small quantity of SO_2 is needed, as in racking wine, and careful methods of burning are used, the weight of sulfur may be taken as a fair measure of the SO_2 produced which may be as high as 85 per cent* of the theoretical amount. When larger quantities are needed, the weight of sulfur used becomes a very uncertain measure of the SO_2 obtained. When the larger quantities are needed, as in the defecation of must, it is interesting to know what is the maximum amount of SO_2 that can be produced in a cask by burning sulfur. By using an excess of sulfur that lost by volatilization and melting does not come into the account.

A cask or other closed space contains a certain amount of oxygen which, if it combined with sulfur would produce exactly twice its weight of SO_2. This we will call the theoretically possible yield which is about .6 grams to 1,000 c.c. of air at 0° C. In practice, the possible yield is much less than this. The difference is caused by losses of oxygen, some of which combines to form sulfuric acid, some escapes from the barrel as the air expands with the rise of temperature and some fails to combine when the proportion in the air becomes too small to support the combustion of the sulfur.

These facts are shown by the results of tests given in the following table:

TABLE No. 1.

SO_2 produced by burning sulfur in a closed space.

Volume of vessel.	Sulfur used.	Sulfur melted.	Sulfur sublimed.	SO_2 formed.	Sulfur utilized.	Oxygen utilized.
1. 3700 c.c.	.180	0.0	.0494	.2613	72.6%	11.6%
2. 3700 c.c.	.520	0.0	.1790	.6830	65.7	30.4
3. 3700 c.c.	.600	.08	.1880	.8241	68.7	36.6
4. 3700 c.c.	1.300	.56	.3050	.8710	33.5	38.7
5. 3700 c.c.	2.290	1.68	.2150	.7900	14.2	35.1

Test No. 1 represents the burning of a little more than 6 ounces of sulfur in a 1,000 gallon cask. Nearly three fourths of the sulfur is oxidized to SO_2 and about one tenth of the oxygen of the air is utilized. As we increase the amount of sulfur used we gradually approach a maximum production of SO_2 which corresponds to about 1.5 pounds to 2 pounds of sulfur in a 1,000 gallon closed cask and utilizes a little

* Laborde.

more than one third of the oxygen present. If we use more than this maximum the excess of sulfur simply melts or sublimes and no more SO_2 is produced unless we renew the air in the cask.

Another element of uncertainty in this method of sulfuring is the failure of the must to take up all the SO_2 produced in the cask. Some of it always escapes with the air forced out of the cask by the incoming must. This loss is increased with the rapidity of filling, it is greater when the must is introduced at the bottom of the cask and when it enters in a solid stream. It is greater usually in small casks than in large. It can be diminished by filling the cask slowly and especially by introducing the must in a spray by means of a rose-nozzle or similar device.

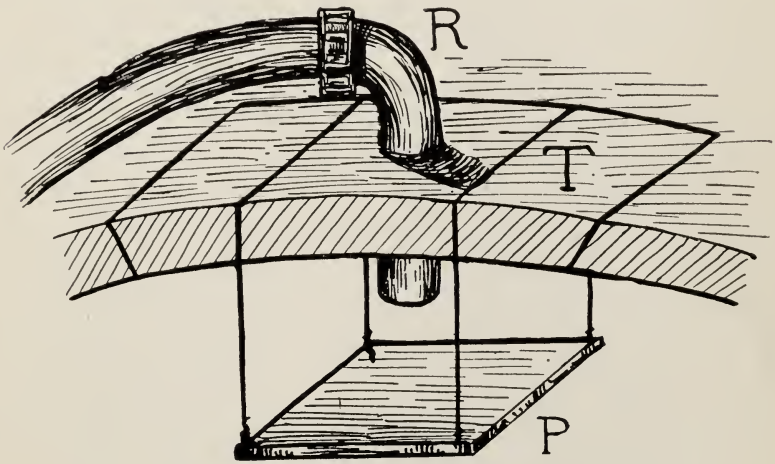


Fig. 4.—Device for spraying must into sulfured cask.

R, hose for entrance of wine; T, cover of manhole to which is attached by cords a small piece of wood, P, on which the entering wine strikes and is broken up into a spray.

In small casks it can be almost eliminated by first introducing a small quantity of must, 5 per cent to 10 per cent of the total, and then rolling the cask until all the SO_2 has been absorbed and filling with the rest of the must.

An idea of the amount of loss by failure of all the oxygen to combine to form SO_2 and by escape of a portion of that formed is given by the following experiments. In wooden barrels of various sizes and in glass flasks holding 2,600 c.c. all the sulfur possible was burned. Each was then filled with water, must or wine, run in slowly. As soon as full, the amount of SO_2 in the liquid in each vessel was determined with the results shown in Table 2.

TABLE No. 2.

Absorption of SO_2 with maximum sulfuring.

Container.	Liquid.	Per cent SO_2 absorbed.	Theoretical yield.	Ratio to theoretical yield.
2600 c.c. flask	Water	.00796	.0546	14.38%
2600 c.c. flask	Water	.00948	.0546	17.34
2600 c.c. flask	Water	.00920	.0546	16.85
2600 c.c. flask	Must	.00535	.0546	9.79
2600 c.c. flask	Must	.00504	.0546	9.23
2600 c.c. flask	Must	.00663	.0546	12.14
2600 c.c. flask	Wine	.0154	.0546	28.51
5 gallon barrel	Water	.0133	.0546	24.17
5 gallon barrel	Water	.0126	.0546	23.07
28 gallon barrel	Water	.0142	.0546	26.01
28 gallon barrel	Water	.0168	.0546	30.76
28 gallon barrel	Water	.0132	.0546	24.17
50 gallon barrel	Water	.0118	.0546	21.43

From Table No. 2 it is seen that wine absorbs the gas more readily than water and water in turn is a better absorber of SO_2 than must. The averages for each in a 2,600 c.c. bottle are: water 88.8 milligrams, SO_2 per liter, must 56.7 milligrams per liter and wine 154 milligrams per liter. The per cent of sulfurous acid absorbed in the barrels was greater on the average than in the glass bottle, owing no doubt to the relatively greater surface exposed in the wooden containers, due to the inequalities of the surface and pores of the wood. The film of water in the pores, etc., absorbs the SO_2 rapidly. The figures indicate that by burning a maximum amount of sulfur in small barrels and running in the liquid by means of a plain hose the must or wine will be sulfured at the rate of 50 to 150 milligrams per liter.

We may reckon roughly that where all ordinary precautions are taken to make the must absorb all the SO_2 produced by burning as much sulfur as possible in a cask, we obtain as a maximum one third of the amount corresponding to the oxygen of the air. This represents a sulfuring of approximately 200 milligrams of SO_2 per liter or .02 per cent, corresponding to an addition of 40 grams of potassium metabisulfite to a hectoliter or about 3 pounds to 1,000 gallons of must. This is about the minimum amount necessary in the defecation of must and requires the burning of about 2 pounds of sulfur in a 1,000 gallon cask.

2. *Liquid Sulfurous Acid.*—Gaseous SO_2 can be liquefied by pressure or by cooling below —10° C., at which temperature it remains liquid at ordinary atmospheric pressure. The liquid is 1.4 times as heavy as water. It vaporizes rapidly at ordinary temperatures unless confined under presure.

Liquid SO_2 is manufactured commercially in Germany from crude sulfur or iron pyrites. The gas obtained by burning the former or roasting the latter is purified by passing through water, which retains

such impurities as sulfuric acid, arsenic, sublimed sulfur and zinc sulfate. The gas is then cooled a few degrees below 0° C. to get rid of the water vapor and finally liquefied by pressure. The pure, dry liquid is then placed in iron cylinders for shipment.

When used in wine-making, some measuring device is necessary. In that constructed by Pacottet, the liquid is forced by the pressure in the cylinder into a small glass measuring tube from which it is allowed to vaporize, through a small copper tube, directly into the must or wine.

The advantages of this method of sulfuring are the great accuracy with which the doses can be measured, the non-corrosive properties of the pure, dry liquid and the absence of such impurities as sulfuric acid, free sulfur and hydrogen sulfide which accompany the SO_2 obtained directly from burning sulfur.

3. *Solutions of Sulfurous Acid.* SO_2 is soluble in water, which dissolves about 69 volumes at 0° C. The gas is lost rapidly with a rise of temperature and at 20° C. water will hold only 36 times its volume and at 40° C. less than 19. This variability of the water solution makes it entirely unsuited for use in wine-making and its corrosive action on metals and its bulkiness make it inconvenient to handle. It is produced cheaply as a by-product of smelters and in some cases might be used as a disinfectant.

SO_2 is more soluble in alcohol, and the solutions, therefore, less bulky. They are, however, also too variable and unstable for use in wine and too expensive for use in disinfection.

4. *Solid Forms; Salts.* Sulfurous acid unites with bases such as potash, soda and lime to form various series of salts of which the principals are the *sulfites, bisulfites,* and *metabisulfites.* These salts all break up with the evolution of SO_2 when they are brought in contact with a stronger acid. The tartaric acid of grape must, for example, will combine with the base and liberate the SO_2, which is then free to exert its action on the must.

The base in combination with the tartaric acid remains in the must or wine. For this reason the soda and lime salts cannot be used, as they would introduce something foreign to the grapes.

The potash salts on the other hand, introduce only bitartrate of potash or cream of tartar, a normal ingredient of the wine. The amount introduced, moreover, is very small, much less than the variations between different samples of grapes. Any of the pure potash salts may be used, but they differ considerably in strength, cost and convenience.

In potassium sulfite, K_2SO_3, the potash is combined with the minimum amount of SO_2, of which it contains approximately 41 per cent. The bisulfite, $KHSO_3$, contains when pure, 53 per cent of SO_2, but it

loses strength rapidly by oxidation. The metabisulfite, $K_2S_2O_5$, is the strongest of all, containing when perfectly pure and fresh, a little over 57 per cent. It is also more stable than the bisulfites. As found in commerce, it contains only from 50 per cent to 54 per cent and in practice it is usual to consider that the salt will yield half its weight in SO_2, which is exact enough for ordinary usages. It will keep fairly constant in composition for months or even years if kept in tightly stoppered glass vessels and in a dry place. For use in wine-making, it should be guaranteed free from all injurious impurities.

It is applied directly in the solid form to the must or made up shortly before using into a 10 per cent solution in water. Water solutions must be made and kept in glass or earthenware vessels, as when concentrated they act very rapidly on metals.

(c) *Transformations of Sulfurous Acid.* The SO_2 which is introduced into the must or wine does not all remain free or active. Some of it unites with the sugar and other components of the liquid and becomes more or less inactive in the combined form. Finally, both the free and the combined forms disappear more or less completely and with more or less rapidity. The "total SO_2" includes both the free and the combined.

1. DISAPPEARANCE FROM VARIOUS SOLUTIONS. To ascertain the extent and rate of disappearance from various solutions the following tests were made:

TABLE No. 3.

Disappearance of SO_2 from various solutions.

Solution.	Time.	SO_2 per liter	Solution.	Time.	SO_2 per liter
Water	0	674 m.g.	Water, plus .5% tartaric acid.	0	674 m.g.
	50 hrs.	630		50 hrs.	542
	92	541		92	395
	169	428		169	277
	288	302		362	68
	362	231		462	40
	390	186			
	462	138			
Water	0	252	Water, plus .5% tartaric acid.	0	271
	26	176		26	207
	74	25		74	143
	91	0		91	134
Fresh must, Balling 20%.	0	200	Raisin must, Balling 24%.	0	200
	24	185		42	197
	39	165		72	192
	113	125		91	166
	135	113		116	134
	543	103		140	102
				193	96
Raisin must, Balling 24%.	0	600	Raisin must, Balling 24%.	0	1500
	18	364		42	1075
	42	320			
	67	262			

Some of the results of Table No. 3 are shown graphically in Diagram No. 1. The line representing the disappearance of the SO_2 from water show that this disappearance is very rapid and almost directly proportionate to the time. The curve of the line of the solution containing tartaric acid is at first steep, gradually becoming flatter. This indicates an increase in the rapidity of loss of SO_2 at first and a decrease later. The tartaric acid seems at first to facilitate the escape of SO_2 and later to oppose it.

The disappearance is much slower in the musts, showing a retarding influence due to the various substances dissolved in it. The retarding influence varies in the different musts. The raisin must, containing

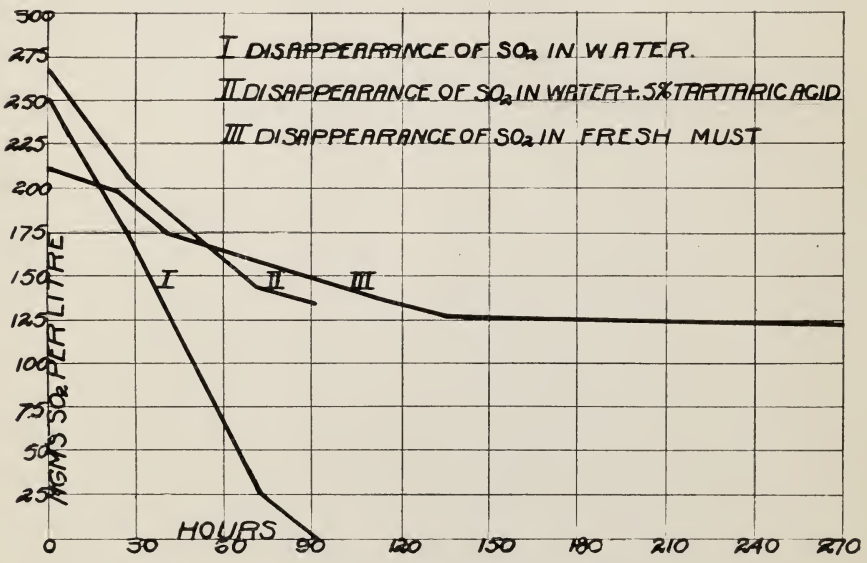


DIAGRAM 1 (see table 3).

Disappearance of SO_2 from various solutions.

more sugar shows, at first, slightly more retarding effect than the fresh must. At the end, however, so far as the tests were carried, the raisin must lost slightly more of its SO_2.

2. DISAPPEARANCE FROM GRAPE MUST. In spite of the superior retarding effect of the raisin must, it commenced to ferment sooner than the fresh and with a larger total amount of SO_2. This indicates a difference in the fermentation restraining power of the SO_2 in the two musts. An explanation of this is given in the following tests showing the changes in the amounts of free SO_2 in various musts.

These tests, shown graphically in diagram 2, show a much slower disappearance of the active free SO_2 from the fresh grape must than from the sweeter raisin must. The results reduced to percentages of free SO_2 remaining at various periods are compared in Table 5.

TABLE No. 4.

Disappearance of free SO_2 from various musts.

Experiment.	Time in hours.	Free SO_2 in milligrams per liter.
No. 215—Fresh grape must, Balling 20%	Added	200
	0	175
	24	97
	39	95
	113	70
	135	61
	168	55
	543	2.5
No. 225—Raisin must, Balling 24%	Added	200
	0	33
	1	30
	6	26
	25	18
	56	12
	80	4
No. 222—Raisin must, Balling 24%	Added	400
	0	100
	24	35
	48	35
	78	32
	102	14
	149	7.8
No. 222—Raisin must, Balling 24%	Added	600
	0	116
	18	59
	42	46
	67	23
No. 222—Raisin must, Balling 24%	Added	1500
	0	269
	18	214
	42	128
	67	58

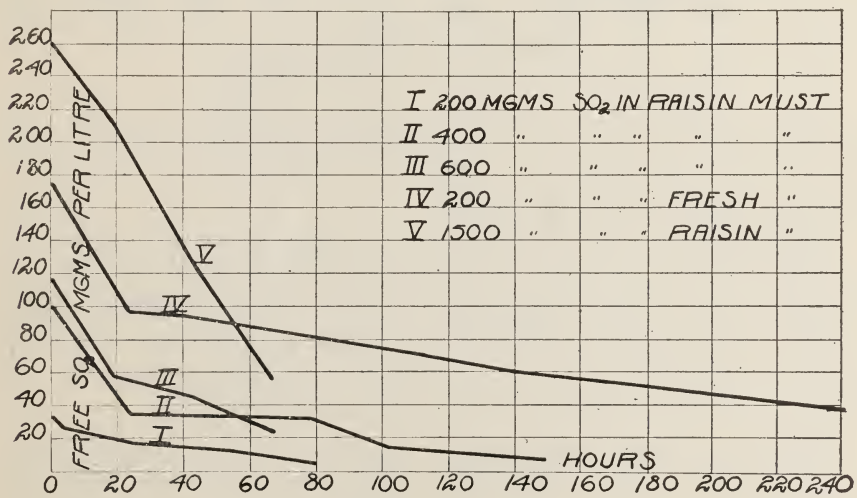


DIAGRAM 2 (see Table 4).

Disappearance of free SO_2 from various musts.

TABLE No. 5.

Free SO_2 remaining in fresh and raisin must.

	Milligrams per liter added.	Per cent remaining.				
		On addition.	36 hours.	67 hours.	113 hours.	168 hours.
1. Fresh must, 20% Balling	200	87.5	47.5	42.0	35.0	24.5
2. Raisin must, 24% Balling	200	16.5	7.5	4.0	--------	--------
3. Raisin must, 24% Balling	400	25.0	8.8	8.3	--------	--------
4. Raisin must, 24% Balling	600	19.3	8.0	3.9	--------	--------
5. Raisin must, 24% Balling	1500	18.0	10.0	3.9	--------	--------

The amount of free SO_2 in the raisin must seems to be a function of the amount added originally. At the end of three days there was about the same per cent of the original amount present in the must which received only 200 milligrams per liter as in that which received 1,500 milligrams. The fresh must at the same time showed ten times as much. That the free SO_2 is the controlling factor in the delay of fermentation is shown by the fact that the raisin must to which 1,500 milligrams was added, started to ferment sooner than the fresh must which received only 200 milligrams. The contrast between the fresh must and the raisin musts is shown clearly in diagram 3.

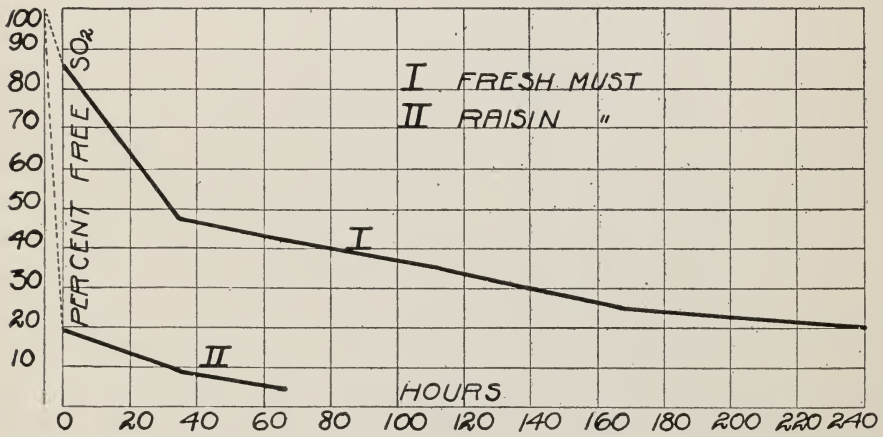


DIAGRAM 3 (see Table 5).—Disappearence of free SO_2 from raisin must and from fresh must.

This great difference might be due to the power of some constituent of the fresh must to set the SO_2 free or of some constituent of the raisin must to hold it in combination. Tests showed that the SO_2 was held equally well in the raisin must when the acidity was raised from .61 per cent to 1.01 per cent, and little difference in this respect could be noticed between raisin musts of 19.5 per cent Balling and 29 per cent Balling. The neutralizing power of the raisin must was found to be

the greater the higher it was heated before the addition, and is apparently due to some undetermined substance formed from the raisin must by the heating.

Differences of neutralizing powers, similar but less marked, have been noticed in practice between different musts, and they indicate one of the reasons for the need of using larger amounts of SO_2 in heavy musts made from grapes growing in hot regions where many dried grapes or raisins are apt to occur.

3. WINERY TESTS. Observations were made in a winery to see how nearly the changes in form and amount of SO_2 found in laboratory tests corresponded with the actual changes in practice.

Three vats of red grapes containing approximately 10 tons each were tested periodically. No. 169 contained Zinfandel grapes from Acampo showing 22.5 per cent Balling, No. 176, the same grapes showing 22.3 per cent Balling and No. 179 Alicante Bouschet grapes from the same locality showing 22.5 per cent Balling.

TABLE No. 6.

Disappearance of total and free SO_2 from red wine vats.

Experiment.	Time in hours.	Balling per cent.	Total SO_2.	Free SO_2.
No. 169—Zinfandel	0.5	22.5	219	127
	49	23.5		93
	64	23.5		90
	74	23.6	188	90
	97	2.5	158	50
	136	0		13
	181	0	126	11
No. 176—Zinfandel	0.5	22.3	75	42
	120	0	66	19
No. 179—Alicante Bouschet	1	22.5	186	65
	7	22.0	169	54
	22	19.5	159	36
	27	15.0	150	30
	34	10.0	131	30
	46	3.5	119	30
	58	0	98	30

From this table we see (Nos. 169, 179) that with an average dose of SO_2 for red wine fermentation (200 milligrams per liter or 12 ounces of metabisulfite per ton of grapes) nearly one half of the total disappears during fermentation. With smaller additions (No. 176) a larger proportion remains.

Reference to the curves of diagram 4 shows that the total SO_2 disappeared rapidly at first and later more slowly and at a fairly uniform rate. The free SO_2 represents at first about 35 per cent of the total,

or 65 milligrams per liter, falling in about seven hours to about 54 milligrams per liter at which point fermentation becomes evident.

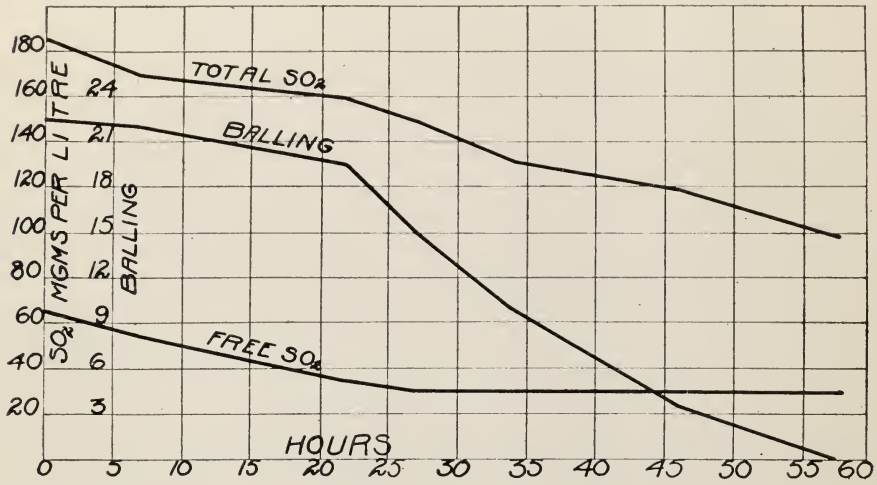


DIAGRAM 4 (see Table 6).

Relation of Balling° to total and free SO_2.

4. DISAPPEARANCE FROM WINE. The changes in form and amount are much less rapid in the wine than in the must. Table No. 7 gives the results of determinations of the free and total SO_2 made at intervals during one to four months after the completion of fermentation. The decrease is comparatively small in all cases. In some cases there is an increase. This is due to racking into sulfured casks by which the supply was renewed.

TABLE No. 7.

Disappearance of SO_2 from wine after fermentation.

Experiment.	Time days after fermentation.	Total SO_2 milligrams per 1.	Free SO_2 milligrams per 1.
No. 169—Zinfandel from Acampo	1	178	35
	4	170	33
	40	115	25
	66	96	26
	112	76	27
No. 170—Zinfandel from Acampo	0	66	19
	74	39	23
	120	40	----------
No. 179—Alicante Bouschet	0	98	30
	60	96	33
	106	108	18
No. 181—Green Hungarian, 1500 gallon tank	2	155	14
	28	150	10
	74	151	13
No. 243—Palomino, 185 gallon puncheon	0	312	10
	46	340	15

5. FINAL FORMS OF SO_2. The change in the form and amount of SO_2 in the fermented wine, as shown in table 7, contrasts noticeably with those which take place in the must before and during fermentation as shown in tables 4, 5 and 6.

When free SO_2 is added to must in the quantities usual in wineries either in the form of gas, liquid or sulfite it decreases very rapidly at first. The total SO_2 also decreases, but with less rapidity. The combined SO_2, on the contrary, increases rapidly at first, the rate of increase gradually slackens, then ceases and finally this form also decreases.

The courses taken by the two forms of SO_2 are shown more or less schematically by diagram 5, which represents an average red wine fermentation in an open vat. The 200 milligrams per liter of free SO_2 added falls rapidly to between 40 and 50 milligrams in about two and one half days, when evident fermentation starts. The point in time and the amount of free SO_2 at which fermentation starts will vary with different conditions of temperature and must composition. During fermentation, lasting in the case supposed about 3 days, the free SO_2 continues to decrease with somewhat diminishing rapidity until it reaches about 25 milligrams per liter which amount remains constant for some time.

In the mean while, the combined SO_2 has been increasing at a slightly slower rate than the decrease of the free. The difference between the decrease of the free and the increase of the combined is shown by the curve indicating the net decrease of the total SO_2.

The final loss of SO_2, shown by the decrease in total, takes place

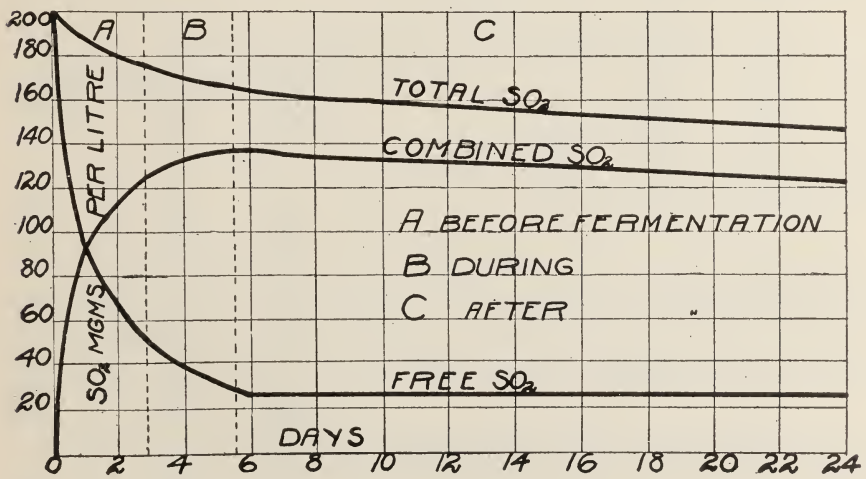


DIAGRAM 5.—Changes in total, free and combined SO_2 in must and wine. A. Ante-fermentation period, about three days. B. Fermentation period, about three days. C. Post-fermentation period.

in two ways. Part is volatilized and escapes into the atmosphere, part is oxidized to sulfuric acid. At first the volatilization is rapid and is the principal cause of the loss shown by the steepness of the curve. The escape of gaseous SO_2 from a recently sulfited vat is readily recognized by its strong odor. During fermentation the decrease becomes slower, owing probably to a slackening or cessation of the vaporization. After fermentation, the decrease is further diminished owing to the cessation of vaporization and a diminution in the rate of oxidation. In the wine after fermentation, the decrease of total and of combined SO_2 is identical. This probably means that as fast as the free SO_2 is oxidized, it is renewed by the setting free of an equivalent amount of the combined forms.

The curves of diagram 5 simply represent general or typical forms which may vary in detail in different cases. For example, the change from the free to the combined form will be more rapid in musts from overripe grapes. The rapidity of loss of total SO_2 may be much greater than indicated if the must is fermented in small vats with abundant aeration, and it may be much slower if fermented in closed casks of large size. The nature, and causes of some of these variations are discussed later.

The final form of all the SO_2 which does not escape into the atmosphere is sulfuric acid. The acid thus produced acts on the cream of tartar giving potassium sulfate and free tartaric acid in small amounts. A measure of the increase in sulfates due to this cause is given by the following tables based on laboratory and winery tests.

TABLE No. 8.

Increase of sulfates due to use of SO_2. (Laboratory tests.)

Experiment.	SO_2 used milligrams per l.	K_2SO_4.	Increase of K_2SO_4.	K_2SO_4 corresponding to total SO_2 added.
No. 138—Sultanina	0	.0259		
	200	.0421	.0162	.0544
	200	.0396	.0137	.0544
	300	.0597	.0338	.0816
	300	.0550	.0291	.0816
Sultanina*	0	.0218		
	10	.0230	.0012	.0027
	50	.0314	.0096	.0136
	75	.0358	.0140	.0204
	100	.0388	.0170	.0272
	150	.0572	.0354	.0408
	200	.0678	.0460	.0544
	250	.0766	.0548	.0680

*Bettoli, R. W. Thesis, 1911.

The increase of sulfates is shown to be approximately proportional to the amounts of SO_2 used, but is in all cases less than the amount corresponding to its total oxidation. Even when large amounts are used, (300 milligrams per liter), the increase is not sufficient to approach the legal limit of 2 grams per liter.

Analyses of wines fermented with SO_2 in the winery gave similar results.

TABLE No. 9.

Increase of sulfates due to the use of SO_2. (Winery tests.)

Experiment.	SO_2 used, milligrams per liter.	Sulfates in unsulfited wine.	Sulfates in sulfited wine.
No. 243—Palomino	600	.0790	.0887
No. 169—Acampo Zinfandel	187	.0527	.0548
No. 176—Zinfandel	185	.0692	.0682
No. 181—Green Hungarian	185	.0446	.0564
No. 173—Petite Sirah	100	.0225	.0749

These tests show that the variations in the sulfates of different grapes and in different samples of the same grape are much greater than the increases due to the use of sulfurous acid.

In the case of the Green Hungarian musts, the original amount of total SO_2 was 185 milligrams per liter. At the time of analysis it was approximately 150 milligrams per liter, indicating a loss of 35 milligrams per liter. The increase in sulfates over the untreated wine was .0118 grams K_2SO_4 equivalent to .00305 grams of sulfurous acid or to 30 milligrams per liter. This indicates that most of the SO_2 in a white wine fermentation disappears by being oxidized to the sulfate of potash. On the other hand, with the red wine, *e. g.*, the Acampo Zinfandel, only a very small amount of the sulfurous acid is oxidized to the sulfate, indicating that most of it disappears as sulfur dioxide gas or is precipitated in the lees as gypsum; that is, sulfate of lime. The latter is probably the case, for when the wines were examined after racking, that is, six weeks after the above analyses the sulfates had decreased very appreciably in all the wines. A few figures will show this plainly.

TABLE No. 10.

Loss of sulfates in wine after fermentation.

Experiment.	K_2SO_3.		
	December 19, 1911.	February 3, 1911.	Loss, per cent.
No. 171—Zinfandel (no sulfite)	.0527	.0343	34.0
No. 169—Zinfandel (sulfited)	.0548	.0590	— 7.7
No. 176—Zinfandel (sulfited)	.0692	.0505	27.1
No. 173—Petite Sirah (sulfited)	.0749	.0590	21.5
No. 179—Alicante Bouschet (sulfited)	.0571	.0559	2.2
No. 182—Green Hungarian (no sulfite)	.0571	.0530	7.0
No. 181—Green Hungarian (sulfited)	.0608	.0560	8.0

(d) *Influence of Composition and Treatment of Must on Fermentation Inhibiting Power of* SO_2. Laboratory tests have shown that fermentation does not start in must to which sulfurous acid has been added until the quantity of free SO_2 has fallen to an amount which is somewhere between 30 and 50 milligrams per liter.

This reduction in the amount of free SO_2 is due to its combination, vaporization and oxidation. It is more or less complete and proceeds with more or less rapidity according to the character of the must and to the treatment to which the must is subjected. Tests were made to determine the influence of various factors on this reduction. The factors investigated were the acid and sugar contents, the presence of pomace and other solid matters, the degree of ripeness of the grapes and the heating of the must to various temperatures.

The tests were conducted in a series of 8 ounce bottles. In each bottle was placed 100 c.c. of must, sulfited to the required degree with a 2 per cent water solution of potassium metabisulfite. The bottles, after inoculation with 1 c.c. of a vigorous culture of Burgundy wine yeast, were kept at a temperature of 30° C. and the start of fermentation determined by the commencement of the evolution of bubbles of gas.

1. ACIDITY. To determine the effect of variations in acidity, a must was used containing 1.18 per cent of total acidity as tartaric. In series *a* the acidity was reduced to .217 per cent by the addition of water, in series *b* to .6 per cent by the same means. In series *c* the acidity was reduced to .63 per cent by means of caustic potash. Series *d* (check) received must without any addition and in series *e* the acidity was increased to 1.8 per cent by the addition of free tartaric acid. In all, the sugar content was made equal to that of the check by means of glucose.

TABLE No. 11.

Influence of acidity of must on effect of SO_2.

Experiment.	Acidity.	Number of hours before the start of fermentation. SO_2 added in milligrams per liter.			
		100.	150.	300.	500.
a	.217	24 hours	69 hours	192 hours	576 hours
b	.600	24 hours	69 hours	240 hours	*
c	.630	48 hours	69 hours	69 hours	96 hours
d	1.180	69 hours	120 hours	*	*
e	1.800	48 hours	192 hours	336 hours	576 hours

*No fermentation.

A diminution of the effect of SO_2 with a decrease in acidity is plainly indicated by a comparison of the check *d* with *a* and *b*, in which the acidity had been reduced by dilution with water. This diminution

is much increased in series *c* where the acidity was reduced by neutralization with caustic potash. In series *a* and *b* the ratio between the free tartaric and the bitartrates to which the acidity is due is the same as in the check series *d*. In series *c* the free tartaric had been changed in whole or part to bitartrate. That indicates that free tartaric acid intensifies the effect of the SO_2 and explains in part the need of larger amounts in overripe grapes in which little free tartaric acid exists. Some other factor, perhaps experimental error—appears to have influenced series *e*, where the addition of free tartaric does not show an intensification of the effect of SO_2 except in the 150 milligram column.

2. SUGAR. The influence of the sugar contents of the must on the effect of SO_2 was tested in another must.

To a series of four flasks containing must of 18.1 per cent Balling was added 100, 150, 300 and 500 milligrams per liter of SO_2. In a parallel series the must was raised to 22 per cent Balling and in another to 26 per cent Balling by the addition of grape sugar. No differences were noted in the time of starting fermentation. The greater resistance of must made from very ripe grapes, therefore, does not seem to depend on the larger sugar contents.

3. LIMPIDITY. The influence of the presence in the must of undissolved solid matters was next investigated. Series *a* represents a red wine fermentation in which the must ferments in contact with the solid parts of the grape. Series *c* represents a clear must such as might be obtained from clean, sound grapes. Series *b* represents a must from inferior grapes or from a continuous press.

TABLE No. 12.

Influence of solid matters of the grape on effect of SO_2.

Experiment.	Number of hours before start of fermentation. SO_2 added, milligrams per liter.			
	100.	150.	300.	500.
a. Must and skins	26	26	26	144
b. Cloudy must	26	26	240	*
c. Filtered must	26	26	*	*

*No fermentation.

This shows that the presence of the skins very much diminishes the effect of SO_2 and indicates that more should be used in the fermentation of red wine than of white. In a cloudy must the effect is also slightly less than in a clear one and shows one reason for the use of larger quantities with must from moldy or damaged grapes.

4. HEATING. The heating of the must or grapes before fermentation is practised to a limited extent, and the following table shows the influence of various degrees of heating on the effect of SO_2. The same must was used in the four series, which were heated to the degrees indicated and then kept at 30° C. The must was heated and cooled before the addition of the SO_2.

TABLE No. 13.

Influence of the heating of must on the effect of SO_2.

Temperature of Heating.	Number of hours before start of fermentation. SO_2 added in milligrams per liter.			
	100.	150.	300.	500.
30° C.	24	69	120	*
80° C., 15 min.	24	46	*	*
100° C., 60 min.	46	46	320	*
120° C., 60 min.	26	26	26	500

*No fermentation.

There seems to be no evidence here that heating to 100° C. has any influence on decreasing the effect of the SO_2. It seems, on the contrary, to have tended to delay the fermentation. This may have been due to the expulsion of the dissolved oxygen which was perhaps insufficiently replenished by subsequent aeration. Heating under pressure to 120° C. for an hour, however, had a noticeable influence on decreasing the effect of SO_2, added subsequently, resembling in a smaller degree the effect of raisins shown in Tables 6 and 14.

5. RIPENESS. The next tests show the influence of various degrees of ripeness. Series *a* and *b* contained musts made from somewhat underripe grapes, series *c* must from ripe grapes and series *d* a must made from raisins.

TABLE No. 14.

Influence of degree of ripeness of grapes on effect of SO_2.

Must.	SO_2 added, milligrams per liter.	Hours before start of fermentation.	
		More than.	Less than.
(a) Unripe Rose of Peru	0	0	25 hrs.
	50	25	46
	100	48	70
	150	70	188
	200	188	280
	300	188	280
(b) Unripe Tokay	0	0	24
	50	24	26
	100	26	46
	150	26	46
	200	46	120
	300	120	280
(c) Ripe Sultanina	0	----------	20
	50	----------	23
	100	----------	44
	150	----------	71
	200	----------	92
	250	----------	139
(d) Raisins (dried Zinfandel)	0	----------	24
	200	----------	24
	400	----------	24
	600	----------	24
	800	24	43
	1000	24	43
	1500	24	43
	2000	43	75

This table shows marked variations in the influence of different grapes in the effectiveness of the SO_2. In the Rose of Peru must 50 milligrams delayed the start of the fermentation as much as 150 milligrams in the Tokay must. The difference between the Tokay and the Sultanina must is not great. The influence of the raisin must in counteracting the effect of the SO_2 is remarkable. An addition of 600 milligrams had no apparent effect on the start of fermentation and 1,500 milligrams had less effect than 150 milligrams in any of the other musts. Even 2,000 milligrams delayed the fermentation only about 50 hours, which is much less than the effect of 200 milligrams in the other musts.

These tests show that to obtain a certain effect in wine-making by the use of SO_2, the amount necessary to use varies very much with various conditions. Very ripe grapes require from two to three times

as much as moderately ripe or underripe grapes while grapes containing raisins may require even larger amounts. About twice as much is necessary in the manufacture of red wine where the must ferments in the presence of the skins as in white wine where the must ferments alone. Musts from moldy or dirty grapes or very cloudy musts from a continuous press require more than clear musts carefully extracted from clean, sound grapes. The heating of the must seems to have little effect, unless excessive, in which case it may necessitate the use of slightly larger amounts of SO_2.

(*e*) *Effects of SO_2 on the Micro-organisms of Must and Wine.* 1. COMPARISON OF THE EFFECTS OF FREE AND OF COMBINED SO_2. It is a recognized fact that the SO_2 which exists free in the must is more active in its effects on micro-organisms than the combined. To obtain data on the relative efficiency of the two forms, pure cultures of a wine yeast were exposed to various amounts of free and to various amounts of combined SO_2 for a period of twenty-four hours. The yeast was then transferred to flasks of must containing no SO_2, and note taken of the cases in which fermentation started. Where fermentation occurred the yeast had evidently not been killed, where it did occur the yeast had been killed or rendered incapable of developing under the conditions of the experiment.

The free SO_2 was obtained by adding various amounts of a solution of metabisulfite to water in which the yeast was placed. The combined form was obtained by adding various amounts of the solution of metabisulfite to raisin must and allowing this must to stand until most of the SO_2 had combined before adding the yeast.

TABLE No. 15.

Comparison of effects of free with those of combined SO_2.

Exposure to free SO_2, milligrams per liter.			Exposure to combined SO_2, milligrams per liter.				
	On addition of yeast, total.	After 24 hours, total.		On addition of yeast.		After 24 hours.	
				Combined.	Free.	Combined.	Free.
a	27	23	*g*	250	4	250	4
b	50	40	*h*	652	11	594	10
c	100	92	*i*	809	14	716	12
d	245	220	*j*	1019	17	979	17
e	481	452	*k*	1286	22	1181	20
f	926	858	*l*	1804	31	1804	31

After an exposure for twenty-four hours to the quantities of SO_2 indicated in the table, the yeast of each flask was removed to flasks of must where the conditions were made as favorable as possible to fermentation.

The yeast from flask *d* produced fermentation but those from flasks *e* and *f* did not. This indicates that an exposure for twenty-four hours to between 250 and 500 milligrams of free SO_2 per liter will kill yeast or render it incapable of development. On the other hand, the yeasts in all the series from *g* to *l* containing combined SO_2 fermented without difficulty, indicating that 1,800 milligrams of combined SO_2, even when accompanied with a small quantity of free, has no effect on the vitality of the yeast.

A closer approximation to the relative efficiency of the two forms was made by further tests of the same character shown in Table 16.

TABLE No. 16.

Comparison of effects of free with those of combined SO_2.

Exposure to free SO_2, milligrams per liter.			Exposure to combined SO_2, milligrams per liter.			
	On addition of yeast, total.	After 24 hours, total.		Amount added, total SO_2.	24 hours after addition of yeast.	
					Combined.	Free.
a	253	178	*g*	2000	1426	118
b	301	230	*h*	3000	2365	277
c	354	250	*i*	4000	3080	330
d	424	307	*j*	5000	3894	542
e	477	477	*k*	6000	4146	896
f	555	550	*l*	7000	5113	826

The yeast from flask *b*, which contained over 230 milligrams of free SO_2, fermented while that of flask *c*, which contained less than 354 milligrams, did not. The amount of free SO_2 which will permanently paralyze yeast in twenty-four hours under the conditions of the experiment, therefore, is less than 354 milligrams and more than 230 milligrams.

The yeast from flask *h*, containing over 2,365 milligrams of combined SO_2, fermented, while that of flask *i*, containing less than 4,000 milligrams, did not. The flasks with combined SO_2, however, contained also smaller amounts of free which must be taken into account in estimating the relative efficiency of the two forms from these figures. Flask *h*, which fermented, contained 277 milligrams of free SO_2. If we take 354 milligrams as the amount of free necessary to kill the yeast as indicated by flask *c*, we may reasonably conclude that free SO_2 corresponding to the difference between this figure and that of 277, the amount of free SO_2 in flask *h*, is more effective than the 2,365 milligrams of combined in the latter flask. The relative efficiency of the two forms in killing yeast seems to be, therefore, somewhere near 77/2365. This indicates that the free SO_2 is more than thirty times as effective in this respect as the combined. This figure, however, must

not be considered as anything more than an approximation. It is quite possible that the true ratio in this respect is very much larger. All that the tests prove is that under some conditions about 300 milligrams of free SO_2 will kill yeast, and that combined SO_2 in much larger amounts than are ever used in practice has no permanent injurious effect on it.

The object of the foregoing tests was to determine the permanent effect of SO_2 on yeast which had been exposed to its action. As was shown, an exposure to about 300 milligrams per liter will kill yeast in twenty-four hours. This may be taken as a measure of its disinfectant power. Its antiseptic power measured by the amount in a nutritive solution which will prevent fermentation is much smaller. The ratio in antiseptic effectiveness between the free and combined is similar to that in disinfectant effectiveness as shown by the following tests.

Wine yeast was introduced into raisin must containing 2,000 milligrams per liter of total SO_2. Determinations of the free SO_2 were made at intervals and the time of the start of fermentation noted.

TABLE No. 17.

Amount of free SO_2 which will permit fermentation to start.

Date.	Total SO_2 added milligrams per liter.		Free SO_2 found milligrams per liter.		Remarks.
	a	b	a	b	
January 1	2000	2000	246	246	Added yeast.
January 10	----------	----------	83	83	No fermentation.
January 17	----------	----------	66	----------	No fermentation.
January 25	----------	----------	32	39	A fermenting; B not fermenting.

In this test the yeast did not become active until the amount of free SO_2 had fallen to between 30 and 40 milligrams per liter. This is probably not a maximum for wine yeast, as the long exposure to large quantities of free SO_2, which occurred at the beginning of this test, probably weakened the yeast and delayed its start.

To compare the antiseptic effect of combined SO_2 a parallel test was made. A series of flasks was filled with a raisin must which had been heated to a high temperature under pressure to cause rapid combination of the SO_2 added later. From 200 to 2,000 milligrams were added to the different flasks which were then inoculated with 1 per cent of a vigorous culture of wine yeast. All the flasks commenced to ferment within three days. This shows that 2,000 milligrams per liter of SO_2 will not prevent fermentation, if most of it is in the combined form and indicates an antiseptic ratio of over 60 to 1 in favor of the free.

2. EFFECT OF SO_2 ON THE MULTIPLICATION OF THE PRINCIPAL MICRO-ORGANISMS OCCURRING ON GRAPE. One of the chief uses of sulfurous acid in wine-making is to prevent the development and activity of injurious organisms entering the fermenting vats with the grapes or from other sources. Experiments were made to determine the relative susceptibility of the principal of these as compared with wine yeast.

Five series, each of 4 small flasks, were filled with normal grape must and to each flask of a series a different amount of SO_2 was added. Each series was then inoculated with a pure culture of a different organism and the number of active cells determined by means of plate cultures. Thirty-six hours later the number of active cells in each flask was determined again in the same way.

TABLE No. 18.

*Effect of SO_2 on multiplication of micro-organisms.**

SO_2 milligrams per liter.	Wine yeast.	Apiculatus.	Wild yeast, Pastorianus form.	Penicillium.	Aspergillus.	Vinegar bacteria.
	Number of cells at start.					
	20,000	150,000	620,000	120,000	450,000	310,000
	Number of living cells after thirty-six hours.					
0						610,000
50	640,000	200,000	580,000	40,000	120,000	14,000
100	2,000,000	75,000	6,000	0	20,000	300
200	310,000	56,000	190	0	30,000	2
400	36,000	0	0	0	0	0

*All the organisms except the wine-yeast were isolated from California grapes.

This table shows very clearly the superior resistance of wine yeast. Fifty milligrams per liter diminished the number of molds and bacteria and allowed only a very small increase of apiculatus. The numbers of the last were decreased by 100 milligrams. The wine yeast increased rapidly with 50 and 100 milligrams and with slightly less rapidity with 200 milligrams. Even with 400 milligrams its increases were greater than that of apiculatus with only 50 milligrams.

These tests indicate that with the must used and under the conditions of the experiment, an addition of 100 milligrams per liter (equal to 26 ounces of metabisulfite to a thousand gallons) the development and activity of all the common injurious micro-organisms would be prevented and a practically pure yeast fermentation insured.

3. EFFECT OF SO_2 ON THE MIXTURE OF MICRO-ORGANISMS OCCURRING NATURALLY ON GRAPES. When grapes are brought to the winery, and are crushed, large numbers of molds and wild yeasts adhering to their surfaces get into the must. Some true wine yeast is usually present on ripe grapes but in smaller numbers. Experiments were

made to test the effect of small additions of SO_2 on such mixtures of micro-organisms. A must from somewhat moldy and damaged Palomino grapes was used, containing consequently a large number of active cells. The must was divided among a number of flasks to each of which a different small quantity of metabisulfite was added.

Before adding the SO_2, a count was made of the number of active cells present. Three hours after the addition, another count was made. Before taking the samples on which the counts were made, the flask was thoroughly agitated so that the count represents the total number of active cells present, none of them having been removed by settling.

TABLE No. 19.

Modification of relative numbers of micro-organisms in natural must.

SO_2 added, milligrams per liter.	Time exposed.	Number of active cells per cubic centimeter.		
		Molds.	Apiculatus.	Yeast.
0	0	97,625	7,341,400	58,575
30	3 hrs.	13,310	189,930	182,995
75	3	19,360	21,780	33,880
120	3	14,650	14,650	43,950
180	3	7,600	0	58,400
240	3	4,800	0	73,600

This shows that the relative susceptibility of the molds, apiculatus and wine yeast are the same in mixtures as in pure cultures. The reduction in the proportions of active undesirable cells is very marked and very rapid even with small amounts. In the untreated must the apiculate yeast constituted 98 per cent of the total number of cells and the wine yeast less than 1 per cent. In three hours after the addition of 75 milligrams per liter (equal to 5 ounces of metabisulfite per ton) this proportion was changed to 28 per cent for the apiculate and 45 per cent for the wine yeast. With 180 milligrams per liter (equal to 12 ounces of metabisulfite per ton) the apiculatus was eliminated and the wine yeast constituted 88 per cent of the total. The small proportion of active mold spores left is negligible, as they usually have no effect on the fermenting wine.

A winery test of a similar nature gave similar results. Zinfandel grapes, which had been transported by rail, were crushed into a 2,200 gallon fermenting vat and sulfited at the rate of 8 ounces of potassium metabisulfite to the ton. A counting of the active cells was made before sulfiting and another an hour after the addition of SO_2. In the untreated must was found:

Molds ---------- 1,600,000 per cubic centimeter = 35.7 per cent.
Apiculatus ---------- 2,830,000 per cubic centimeter = 63.2 per cent.
Wild yeasts ---------- 30,000 per cubic centimeter = .7 per cent.
Wine yeast ---------- 20,000 per cubic centimeter = .4 per cent.

In the counting plates made after sulfiting only wine yeast was found and in increased numbers. All the other kinds had disappeared or failed to develop. This shows plainly the effectiveness of moderate doses of SO_2 in paralyzing injurious organisms. It is possible that some of the apiculatus and wild yeast might have recovered in time if afforded the opportunity, but if a starter of good yeast were used soon after sulfiting the fermentation would be over and the wine safe before they could develop.

4. EFFECT OF SO_2 IN DEFECATION. In making white wine, it is a common practice, after separating the must from the pomace by draining and pressing, to allow it to clear by settling and then to separate it from the sediment by drawing off into clean vats. This practice is known as "defecation," and is possible only when fermentation fails to start within about forty-eight hours. This delay of fermentation is usually insured by means of sulfuring or sulfiting.

Defecation removes most of the suspended inert matters such as fragments of the grape, and soil particles. It removes also a part of the living cells of micro-organisms. When sulfurous acid is used we obtain, in addition to this mechanical separation, the paralyzing effect already discussed. Tests were undertaken to determine the relative part of each of these factors in the results.

ELIMINATION OF INERT SOLIDS. Laboratory tests by R. W. Bettoli* demonstrated the difficulty of defecation by means of refrigeration except with musts of exceptionally clean and sound grapes which need it least. Certain molds and wild yeasts grow at very low temperatures and produce sufficient fermentation to prevent an adequate settling of impurities. Previous pasteurization is even less effective, at least with the musts used, on account of the increase in the viscosity of the liquid due to changes in the pectic matters which prevented settling.

Where SO_2 was used at the rate of 150 milligrams per liter the defecation was complete, the supernatant liquid being perfectly clear to the eye and containing no suspended matter which could be weighed after passing the clear must through a gooch filter. The original must tested by weighing a gooch filter through which it had been passed showed .554 per cent of suspended floating matter. This is sufficient to cause a bulky sediment in a wine made from undefecated must. The presence of so large a bulk of impurities during fermentation can hardly fail to have an injurious effect on the odor, taste and finer qualities of the wine.

These tests give us no measure of the extent to which elimination of the cells of micro-organisms is carried by defecation. Many of

*Loc. cit.

these cells will pass through an ordinary filter, and even if they were all retained their weight is too small for determination. Their elimination can be determined only by cultural and microscopical methods.

Bettoli* has shown that the number of active cells in a must can be reduced by defecation with from 100 milligrams to 300 milligrams of SO_2 per liter from 1,240,853 per c.c. to 255 per c.c. at 22° C. and to 1,500 per c.c. at 28° C. in forty-eight hours. The efficiency of the smallest amount of SO_2 was very little inferior in this respect to that of the larger. The lower efficiency at the higher temperature probably represents a slight mulplication of the cells which were not eliminated.

These results show the total reduction of active cells due both to settling and to the paralyzing effect of the SO_2. A measure of the part of each of these actors is given by comparison with determinations of the numbers of active cells in the sediments formed. In the sediments the average number of active cells was found to be 615 per c.c. at 22° C. and 7,747 per c.c. at 28° C. This indicates that the greater part of the cells were rendered inactive by means of the SO_2 and that only a relatively small number were removed by actual settling. The results are shown in more detail in Table 20.

TABLE No. 20.

Elimination of micro-organisms by defecation.

Number of active cells originally in must 1,240,853 per cubic centimeter.

SO_2 used, milligrams per liter.	Temperature C.	Number of active cells per cubic centimeter.		Elimination per cent.	
		In sediment.	In clear juice.	Sediment.	Clear juice.
100	22	1,005	45	99.91	99.99
150	22	645	630	99.94	99.94
200	22	585	315	99.95	99.97
300	22	225	30	99.98	99.99
100	28	16,857	3,810	98.64	99.69
150	28	5,055	180	99.59	99.98
200	28	6,045	1,695	99.51	99.86
300	28	3,030	345	99.75	99.97

This table shows that on the average 99.92 per cent of the active cells were eliminated from the clear must and 99.56 per cent from the sediment, leaving only .08 per cent in the former and .44 per cent in the latter capable of immediate development. The tests, however, do not show the nature of the cells which remain, a matter of importance.

ELIMINATION OF MOLDS AND WILD YEASTS. Laboratory experiments with apple juice showed that defecation with 100 milligrams of SO_2 per liter eliminated in twenty-four hours all active micro-organisms in

*Loc. cit.

both the clear juice and the sediment except the true yeasts. All the true yeasts were eliminated from the clear juice when 300 and 400 milligrams per liter were used. In the sediment active true yeasts were found in small numbers even when 400 milligrams per liter were used.

These tests indicate that the unfavorable activities of injurious organisms can be completely prevented by the use of SO_2 alone without defecation by settling. The utility of the settling is simply to remove the solid impurities which would depreciate the quality of the wine if they remained present during fermentation. Except perhaps with very inferior grapes, this result is of much less importance than the prevention of the activities of injurious organisms. Defecation by refrigeration is therefore much inferior in its effects to defecation by means of SO_2; in other words, the sulfiting of the must is of more importance than the clearing by settling. When sulfiting alone is used, after a delay of more or less extent, depending on how much SO_2 is used, the must will finally go through a practically pure fermentation; that is, the true, natural wine yeasts present will take exclusive possession of the must.

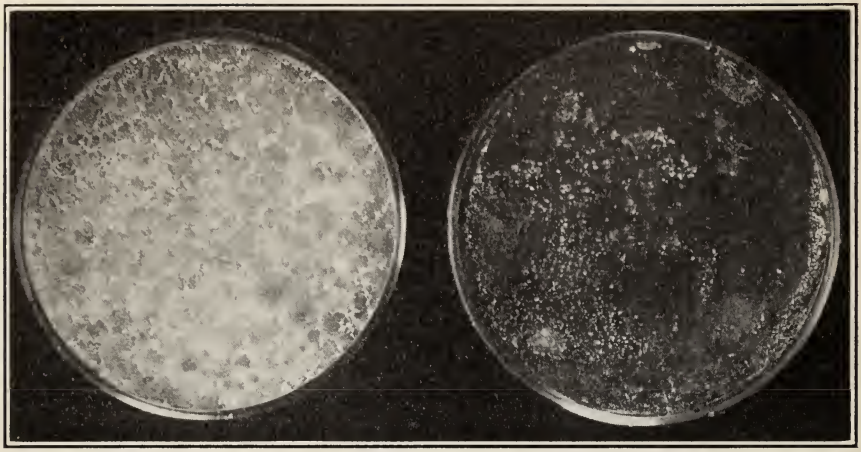
When the clear sulfited must, however, is drawn off the sediment before the start of fermentation there may be little or no yeast present. In this case the delay of fermentation may be very great or, worse still, it may be due to micro-organisms which get into the must after defecation and these micro-organisms are very likely in many cases not to be wine yeast principally. Whenever sulfiting and defecation are both practised, a starter, preferably of pure yeast, should be used immediately after removal of the must from the defecating vat.

Observations in the winery corroborate these results obtained in the laboratory. A must made from Green Hungarian grapes shipped from the San Joaquin Valley was defecated in a 1,500 gallon vat. The must was allowed to settle after adding metabisulfite at the rate of 6 ounces to 100 gallons. The number of molds, apiculatus and wine yeast cells was determined in the must before sulfiting and in the cleared must after settling thirty-two hours, with the results shown in Table 21.

TABLE No. 21.

Elimination of micro-organisms in a 1500-gallon defecating vat.

	Molds.	Apiculatus.	Wine yeast.
Original must	29,262 per c.c.	6,603,028 per c.c.	29,262 per c.c.
Defecated must	256 per c.c.	0 per c.c.	492 per c.c.
Elimination	91.3%	100%	83.2%



A B

FIG. 5.

A, plate from undefecated must, showing molds; B, plate from defecated must, showing yeasts.

A similiar test was made with a Palomino must of the same origin. This must was defecated in the yeast apparatus with 4 ounces of metabisulfite to 100 gallons.

TABLE No. 22.

Elimination of micro-organisms in small vat.

	Active cells per cubic centimeter.		
	Molds.	Apiculatus.	Wine yeasts.
Original must	112,658	1,565,658	529,133
Defecated 8 hours	44	660	13,800
Defecated 20 hours	10	0	16,280
Elimination at 8 hours	99.96%	99.96%	97.4%
Elimination at 20 hours	99.99%	100.00%	97.0%

These results show that comparatively small amounts of SO_2 very rapidly eliminated all the active cells except those of wine yeast. For the fullest effect, desirable in the preparation of starters, defecation should continue for at least twenty-four hours. This will insure the absence of all active injurious organisms. There will probably be a slight admixture of the natural wine yeasts of the grape when selected pure yeasts are used, but this contamination is in practice perfectly harmless and experience with the use of selected pure yeasts in wineries shows that with ordinary precautions it is reduced to very little.

5. CHANGES IN THE SUSCEPTIBILITY OF WINE YEAST TO SO_2. In 1905 a series of experiments was made to test the alleged possibility of training a yeast to withstand increased amounts of SO_2. A must of 20 per cent Balling and .7 per cent acidity was divided among six flasks, to each of which was given a different dose of SO_2. They were then all inoculated with a pure culture of wine yeast. The flask containing the least SO_2 commenced to ferment first. As soon as this occurred, the flask containing the next higher amount was inoculated with yeast from the fermenting flask and so on until the end of the series. The results are shown in the following table:

TABLE No. 23.

Exposure of yeast to increasing doses of SO_2.

Flask.	SO_2 added, milligrams per liter.	Remarks.
(*a*)	60	*April 16, all flasks inoculated.*
(*b*)	100	*April 19, (a) fermenting, inoculated (b) from (a).*
(*c*)	200	*April 20, (b) fermenting, inoculated (c) from (b).*
(*d*)	350	*April 21, (c) fermenting, inoculated (d) from (c).*
(*e*)	550	*April 23, (d) fermenting, inoculated (e) from (d).*
(*f*)	1000	*May 1, (e) fermenting, inoculated (f) from (e).*
		May 8, (d) fermenting.
		May 15. All flasks fermented dry.

Observations of this kind have been interpreted as showing an increased resistance of yeast to SO_2 when gradually accustomed to its presence. The yeast, for example, in flask *f* receiving 1,000 milligrams of SO_2 per liter had not caused fermentation at the end of fifteen days. When yeast which was causing fermentation in flask *e* to which had been added 550 milligrams of SO_2 was placed in flask *f* fermentation started in seven days.

The correct interpretation of these facts has been shown by later tests to be this: The yeast placed in flask *f* immediately after the addition of 1,000 milligrams of SO_2 was killed or weakened because a large part of the SO_2 was in the free form. Fifteen days later, when the second inoculation of yeast was made, most of the SO_2 had changed to the combined form and insufficient free SO_2 existed to prevent fermentation. The start of fermentation, therefore, was not due to a greater resistance of the yeast, but to decrease of the antiseptic properties of the SO_2.

Other experiments made at the same time pointed to an actual weakening of the yeast by exposure to SO_2. To investigate this point, a series of tests was made in 1911. Three series of nine flasks each were filled with grape must. Each flask of a series received a different

amount of SO_2, the corresponding flasks of each series receiving the same amount. After sulfiting, each flask was inoculated with 1 per cent of the same vigorous pure yeast, unaccustomed to sulfurous acid.

Series III consisting of flasks *a″* to *i″* was left without further treatment until the end of the experiment. Series II consisting of flask *a′* to *i′* and Series I consisting of flasks *a* to *i* were left for five days until it was apparent that fermentation had started in all the flasks where it was possible without new inoculation. In twenty-four hours the first five flasks of all series had commenced to ferment. At the end of five days none of the last three had commenced. In Series II, flask *g′* was then inoculated with yeast from flask *f′* of the same series, accustomed to SO_2. In Series I flask *g* was inoculated afresh with the yeast unaccustomed to SO_2. This process was continued until all the flasks of Series I and II had fermented. Each flask of Series II was inoculated with yeast from the flask immediately below it, while at the same time the corresponding flask of Series I was inoculated with the original yeast unaccustomed to SO_2. The same variety of yeast was used in all cases.

TABLE No. 24.

Comparison of accustomed with unaccustomed yeast.

Series I.	Series II.	Series III.	SO_2, milligrams per liter.
a	*a′*	*a″*	200
b	*b′*	*b″*	250
c	*c′*	*c″*	300
d	*d′*	*d″*	350
e	*e′*	*e″*	400
f	*f′*	*f″*	500
g	*g′*	*g″*	600
h	*h′*	*h″*	800
i	*i′*	*i″*	1000

July 31, 6 p. m. Inoculated all flasks and placed at 29° C.
August 1, 8 a. m. All flasks from beginning to *f*, *f′*, and *f″* fermenting.
August 5, 5 p. m. No flask started above the *f* row in any series.
Inoculated *g* with unaccustomed yeast.
Inoculated *g′* with yeast from flask *f′*.
August 7, 8 a. m. *g* fermenting; *g′* and *g″* not started.
August 8, 8 a. m. *g* fermenting vigorously; *g′* just starting; *g″* not started.
August 10, 8 a. m. *g* and *g′* fermenting vigorously.
August 16, 8 a. m. Inoculated *h* with unaccustomed yeast.
August 16, 8 a. m. Inoculated *h′* with yeast from *g′*.
August 21, 8 a. m. *h* and *h′* fermenting vigorously; *g″* not started.
Inoculated *i* with unaccustomed yeast.
Inoculated *i′* with yeast from *h′*.
August 22, 8 a. m. *i* fermenting; *i″* not started; *i′* not started.
August 23, 8 a. m. *i* fermenting vigorously; *i′* just starting.
g″, *h″* and *k″* not started.

This experiment shows conclusively that exposure to SO_2 does not increase the resistance of yeast, but on the contrary makes it more susceptible. This is shown by the fact that flask *g* inoculated with yeast unaccustomed to SO_2 fermented in fifteen hours, while the corresponding flask *g'* containing the same amount of SO_2 and inoculated with a yeast accustomed to SO_2 did not start to ferment until after thirty-seven hours; and flask *i* inoculated with unaccustomed yeast started to ferment in twenty-four hours, while *i'* with accustomed yeast required forty-eight hours.

None of the last three flasks of Series III started at all, though the free SO_2 must have fallen as low as in the corresponding members of the other series where fermentation took place. This indicates that the yeast was killed or much weakened by the amount of SO_2 placed in these flasks.

Another series of experiments was made in the attempt to make a quantitative determination of the effect on yeast of prolonged contact with SO_2.

A flask B containing 100 c.c. of must, to which 100 milligrams of SO_2 had been added was inoculated with 1 per cent of a culture of Burgundy yeast. A similar flask A without SO_2 was inoculated with the same yeast. Both were incubated at 32° C. When both were in full fermentation, a flask of must containing 100 milligrams of SO_2 per liter was inoculated with the yeast from flask B grown in the presence of 100 milligrams SO_2 and a similar flask was inoculated with yeast from flask A grown in must free from SO_2. The progress of the fermentation was then determined by periodical weighing of the flasks as shown in Table 25. The same procedure was followed in Series II and III using 200 and 300 milligrams of SO_2 per liter, respectively, both for the previous training of the yeast and for the fermentations.

TABLE No. 25.

Increase of susceptibility of yeast by exposure to SO_2.

Series I. 100 milligrams SO_2 per liter. Experiment 372a. Weight in grains.

Hours.	Flask A, untrained yeast.	Flask B, trained yeast.	A—Loss in weight.	B—Loss in weight.
0	304.50	307.69	0	0
23	303.89	307.10	.6	.59
52	301.57	304.64	2.93	3.05
70	299.85	302.80	4.65	4.89
92	297.05	300.07	7.45	7.62
119	295.30	298.50	9.20	9.19
138	294.54	297.79	9.96	9.90
168	294.45	297.65	10.05	10.04
Series II. 200 milligrams per liter. Experiment 372b.				
0	321.05	313.25	0	0
17	320.07	313.10	.18	.15
51	319.10	312.05	1.95	1.20
78	315.77	309.80	5.28	3.45
97	315.24	308.47	5.81	4.78
127	313.05	306.25	8.00	7.00
150	311.81	304.75	9.24	8.50
173	311.10	303.67	9.95	9.58
193	310.75	303.02	10.30	10.25
211	310.42	302.57	10.63	10.68
235	310.20	302.27	10.85	10.88
Series III. 300 milligrams per liter. Experiment 372c.				
0	288.35	287.92	0	0
22	287.83	287.72	.52	.2
45	286.72	286.82	1.63	1.1
65	284.55	284.82	3.80	3.1
83	282.50	282.72	5.85	5.20
107	280.47	280.82	7.88	7.10
141	278.31	278.92	10.04	9.00
165	277.59	277.85	10.76	10.07
182	277.19	277.35	11.16	10.57
227	276.55	276.67	11.80	11.25

In the flasks containing 100 milligrams per liter, the fermentations follow almost exactly the same course, showing no effect from the previous exposure to SO_2. In the flasks containing 200 milligrams the fermentation in the flask inoculated with "trained" yeast is slower at first but catches up by a more rapid fermentation near the end. In the flask containing 300 milligrams the fermentation of the "trained" yeast is uniformly slower from beginning to end.

Both yeasts caused a slower fermentation in the presence of 200 and 300 milligrams of SO_2 than in that of 100 milligrams.

With regard to the rate of fermentation, therefore, we have two effects due to SO_2, both tending to retard it. The fermentation is slower in musts containing the larger quantities of SO_2, owing to the immediate effects of the SO_2 in decreasing the activity of the yeast. This decrease of activity, moreover, persists, so that a yeast which has developed in the presence of SO_2 is less active than before exposure when placed in musts containing large doses of SO_2. These effects are shown in diagram 6.

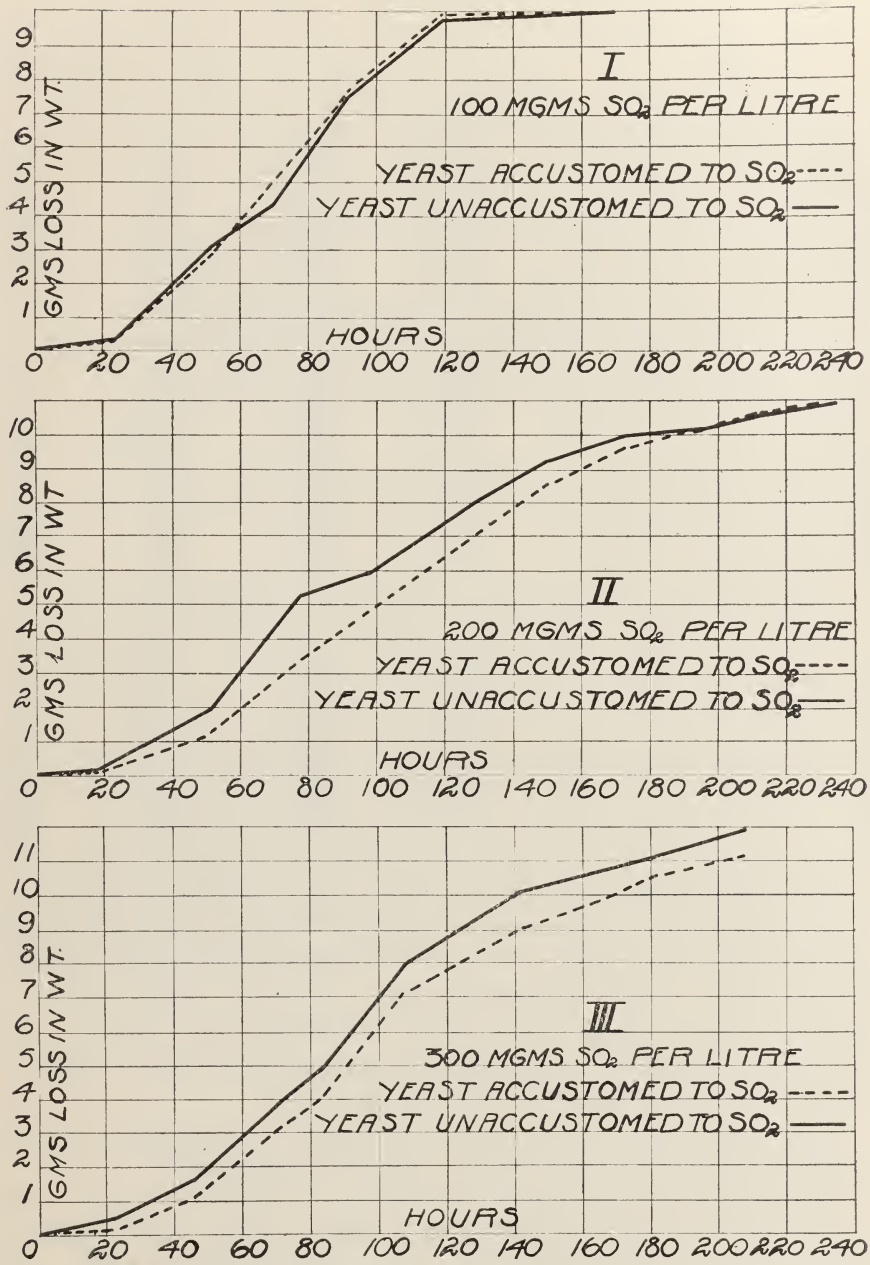


DIAGRAM 6.

Comparison of fermentative activity of "trained" and "untrained" yeast.

The theory that yeast can be gradually "trained" to withstand increasing doses of SO_2 is incorrect under the conditions covered by these tests. On the contrary, its susceptibility, as evidenced by the rate of fermentation, is increased.

This decrease in rapidity may be an advantage in aiding cool fermentation for as indicated by these tests it is principally at the start. In practice it is found, however, that little aid is to be obtained in this way in avoiding hot fermentations. The lesson to be drawn from these tests is that pure yeast and starters should be grown in the presence of comparatively small amounts of SO_2, in order to maintain the vigor and power of rapid multiplication necessary to permit them rapidly to outnumber all other micro-organisms occurring in the must.

Tests made with yeast from a cellar yeast-apparatus gave similar results. Must showing 25° Balling was placed in two series of flasks. Series A consisted of flasks *a, b, c, d* and *e,* which received 100, 150, 200, 300 and 500 milligrams of SO_2 per liter, respectively. Series B consisted of five flasks a_2 to e_2 sulfited in the same way. Series A was inoculated immediately with a vigorous yeast grown in must without SO_2. Series B was inoculated at the same time with the same variety of yeast which had been grown for a month in the yeast apparatus in must sulfited to the extent of 200 milligrams per liter. The yeast in the apparatus was pure and apparently vigorous. Moreover, it had given excellent results in fermenting wine on a practical scale. The course of fermentation in the various flasks was observed by daily readings of the Balling per cent.

Of the flasks inoculated with "untrained" yeast, 3 fermented, but only one of the flasks receiving "trained" yeast. The course of the fermentation is shown in Table 26.

TABLE No. 26.

Weakening of yeast by exposure to SO_2.

Hours.	A—Unaccustomed yeast. Balling per cent.			B—Accustomed yeast. Balling per cent.
	a 100 milligrams.	b 150 milligrams.	c 200 milligrams.	a_2 100 milligrams.
0		25.25	25.25	25.25
96	25.25	25.25	25.25	25.25
120	25.00	25.25	25.25	25.00
145	24.00	25.25	25.25	25.25
168	18.00	24.00	25.00	16.00
193	13.50	20.00	21.00	11.50
221	6.00	10.50	14.00	5.00
240	3.50	7.00	8.50	2.50
268	.50	4.00	5.00	0
292	0	.5	1.0	0
315	0	0	0	0

Here, as in the former series of experiments the accustomed and the unaccustomed yeasts gave almost identical results in the must containing only 100 milligrams SO_2 per liter. Where larger amounts were used, however, the accustomed yeast showed an increase of susceptibility by failing to grow in musts containing 150 and 200 milligrams per liter in which the unaccustomed yeast grew readily.

In these experiments the yeast was added soon after the addition of the sulfite while a large amount of the SO_2 still existed in the free state. In practice, the addition of yeast should be deferred for at least twelve hours after sulfiting, especially where more than 100 milligrams per liter of SO_2 is employed. If this precaution is taken, there need be no fear that the yeast will lack vigor if it has been grown in the presence of only moderate amounts of SO_2.

III. UTILITY AND METHODS OF APPLICATION OF PURE YEAST IN WINE-MAKING.

(a) *Present Status of Pure Yeast in Wine-making in California.* In 1893 the station commenced to investigate the possibility of improving our wines by the use of pure and selected yeast. The first tests with a Johannisberg wine-yeast from Geisenheim on the Rhine were made on a small scale at the station cellar. The results were promising and in 1895 a number of pure yeasts from the Rhine, Médoc, Burgundy, Sauternes, Italy and Algeria were tested at the station and distributed to wine-makers in various parts of the state for trial.

The conclusions drawn from the small station tests were that the use of pure yeast promoted a quicker fermentation, a more prompt clarification and produced a wine of cleaner taste and with some improvement in bouquet and flavor. The tests at wineries were less favorable. In many cases, where the pure yeasts were used, the wines failed to ferment to dryness, while the naturally fermented wines became quite dry. This was especially marked in the case of the German yeast which produced a dry wine only in one case. The successful fermentation was that of a light Burger must and the resulting wine was much superior to the witness. The cause of the failures of this yeast was its unsuitability to our heavy musts. The failure of the other yeasts seems to have been due to the fact that the addition of yeast promoted a rapid development of the fermentation, and a rise of temperature to a point that paralyzed the yeast before the sugar had all been eliminated.

These results showed that a proper selection of yeast was necessary and that the use of pure yeast without some means of controlling the temperature of fermentation was liable to do more harm than good.

Since 1895 the work of testing pure yeast and the methods of application have been continued. Many yeasts from most of the chief wine-producing regions have been imported and tested in comparison with yeasts isolated from Californian grapes and wines. The results have shown that before using pure yeast some means of controlling the injurious yeasts, molds and bacteria must be adopted. This means has been found in the proper use of sulfurous acid. With this help the improvements obtained in the small scale station tests can be realized in practice. All the yeasts tested, both imported and native, have given good results with the exception of some German yeasts which were unsuited to heavy musts. On the whole, the yeasts obtained from the Champagne and Burgundy districts have been found the most suitable and have given the greatest improvement in increase of quality and in simplifying the handling of the wine. For this reason we have limited our supply of yeast to wineries to two or three forms originating in these districts, and they have given satisfactory results in all kinds of wine.

Whether other yeasts will give better results in special cases can be determined only with time and the multiplication of experiments. The improvement obtained by the use of these yeasts is so much greater than any differences existing between different pure yeasts, that it seems safer to use them exclusively in our large scale tests until the methods and advantages of the use of pure yeast are better understood by most cellar-men.

(*b*) *Organisms found on Californian Grapes.* As pointed out in Bulletin 213, the spores of any organism carried by the wind may be found on the surfaces of grapes as they come to the winery. Many of these organisms are incapable of growing on grapes or in must or wine. A large number, however, are capable of growing on the grapes when the conditions are favorable and many will grow in the must and some in the wine itself. They are all useless with the exception of the wine yeast and many of them highly injurious in wine-making. The principal injurious forms have been described in Bulletin 213. Some of the most troublesome often occur in abundance and always in much larger numbers than the necessary wine yeast. In fact, the latter is often present in such small numbers that it cannot be found by ordinary methods.

The possibility of restraining the activities of these troublesome organisms in wine-making by the use of SO_2 has already been discussed. Its necessity is made evident by investigations, made during the past season, on the kinds and numbers of micro-organisms occurring naturally on Californian grapes as they hang in the vineyard

and as they arrive at the cellar after gathering, hauling and shipping. The results emphasize the need of the use of SO_2 and the utility of the employment of selected yeast.

1. Davis. On a sample of Muscat grapes gathered in the vineyard of the University Farm at Davis were found in large numbers, 3 molds, 3 wild yeasts, and, in small numbers, 2 wine yeasts.

TABLE No. 27.

Micro-organisms found on grapes at Davis.*

1. Green mold (*Penicillium* sp.).
2. Gray mold (*Mucor* sp.).
3. Black mold (*Aspergillus* sp.).
4. Apiculate yeast (*Saccharomyces apiculatus*).
5. Film-forming wild yeast (*Saccharomyces* sp.).
6. Sediment wild yeast (*Saccharomyces* sp.),
7. Wine yeast (2 forms) (*Saccharomyces ellipsoideus*).

This is typical of what may be found on any ripe grapes in any vineyard in California or, in fact, in any region. If the grapes are gathered carefully while sound, hauled to the winery promptly and properly handled by the wine maker, the three molds would do no harm. If the grapes are injured by insects, diseases, rough handling or rain, if they are kept for a day or two before being crushed, these molds would very much depreciate their value and even spoil the wine made from them.

The apiculate and other wild yeasts may render the wine inferior by developing in the fermentation vats and casks unless SO_2 is properly used. The wine yeasts from such grapes as these will sometimes not develop at all or not until after the others have increased sufficiently to seriously injure the quality of the wine. This is what often occurs with the first grapes of the season. As the season advances, the wine yeast gradually increases in the grape boxes, crushers and conveyers so that when SO_2 is used to restrain the injurious yeasts, the natural wine yeasts ultimately prevail. This method of obtaining our wine yeast from dirty crushers is, however, uncertain and illogical. With grapes from some localities, it usually gives us finally a sufficiently good yeast, with others it is more uncertain. In all cases the kind of wine yeast we finally obtain is liable to vary from year to year, and it is much better practice to start at the beginning of the vintage with a pure culture of a suitable and tested yeast.

The apiculate yeast found on the Davis grapes was tested and produced a slow fermentation, which at the end of three weeks had pro-

* These observations refer only to such micro-organisms as were present in notable numbers, *i. e.*, usually hundreds or thousands in every drop of must and which grew readily in must.

duced only .3 per cent of alcohol. To do this, it destroyed 2.1 per cent of sugar, from which a wine yeast would have produced over 1 per cent of alcohol. The must, after fermentation, had a fruity odor, and cleared very slowly. It thus showed two of the chief defects of apiculatus fermentation, viz., waste of sugar and persistent cloudiness of the wine.

The film-forming yeast gave a heavy surface growth and very little fermentation. The liquid acquired a disgusting odor and taste and lost 4.3 per cent of sugar with the production of only .3 per cent of alcohol.

The second variety of wild yeast made a heavy sediment growth and caused a short and feeble fermentation. It gave the must at first a fruity odor and after several weeks a decidedly rancid odor and taste, due probably to the formation of butyric acid. It produced only 2 per cent of alcohol.

The two varieties of wine yeast gave a vigorous fermentation, fermenting to dryness a must showing 28 per cent Balling. They were of similar appearance under the microscope, but one gave a very fine-grained pasty sediment, while the sediment of the other was flocculent and more bulky. They were isolated only at the end of the spontaneous fermentation of the must and were not found on the fresh grapes, showing that they were present in much smaller numbers than the injurious forms.

2. Contra Costa County. The principal micro-organisms of grapes grown and crushed in Contra Costa County were isolated from the must as it came from the crusher. The following forms were found:

TABLE No. 28.

Micro-organisms found in must of Contra Costa County grapes.

1. Green mold (*Penicillium* sp.)
2. Gray mold (*Mucor* sp.)
3. Black mold (*Aspergillus* sp.)
4. Apiculate yeast (*Saccharomyces apiculatus*).
5. Wild yeast growing rapidly in must, forming a thick leathery film but causing little fermentation.
6. Wild yeast with large oblong cells forming a thin film, thick sediment and causing little fermentation.
7. Wine yeast forming a fine-grained pasty sediment and causing a good fermentation.

3. Acampo. Grapes grown at Acampo and shipped to Martinez showed a similar choice selection of forms:

TABLE No. 29.

Micro-organisms found in must of grapes from Acampo.

1. Green mold (*Penicillium* sp.).
2. Gray mold (*Mucor* sp.).
3. Gray mold (*Botrytis* sp.).
4. Black mold (*Aspergillus* sp.).
5. Apiculate yeast (*Saccharomyces apiculatus*).
6. Wild yeast—Spherical, film-forming
7. Wild yeast—Large, oblong, film-forming } (*Saccharomyces* sp.).
8. Wild yeast—Elongated, film-forming
9. Wine yeast—Small, ellipsoidal (*Saccharomyces ellipsoideus*).
10. Wine yeast—Spherical (*Saccharomyces ellipsoideus*).

Some of the musts examined were extracted from clean, sound grapes, others from grapes in a more or less broken or moldy condition. Practically, the same kinds of organisms were found in all, and in all cases the wine yeasts were in a very insignificant minority. The condition of the grapes made little difference in the relative number of wine yeast cells present, but influenced considerably the total number of all kinds.

To give a quantitative idea of the relative number of the various forms in various musts, counts were made in the must as it came from the crusher. The method used was to count the number of colonies, which developed from a measured quantity of must in plates of gelatin must.

TABLE No. 30.

Number of micro-organisms in various musts.

Number of active cells in 1 cubic centimeter.

Must.	No. 198.	No. 199.	No. 194.	No. 261.	No. 202.
Molds	59,000	1,300	29,000	98,000	1,600,000
Wild yeasts (plus apiculatus)	28,000	1,267,000	8,603,000	7,341,000	2,880,000
Wine yeasts	0	0	29,000	59,000	20,000

No. 198 was Zinfandel grown in the neighborhood and crushed in good condition. It shows the smallest total number of active cells. All that developed, however, were injurious molds and wild yeasts. This does not mean necessarily that no true wine yeast was present. It simply means that, if there, it was in such small amounts that the methods used failed to reveal its presence.

No. 199 was Alicante Bouschet grown at Acampo and shipped to Martinez. When crushed they were in fairly good condition. The number of mold cells was very small, but that of the wild yeasts high;

owing probably to development and multiplication on broken berries in transit. The wine yeast was again insufficiently numerous to be found.

No. 194 was Green Hungarian grown in San Joaquin County and shipped to Martinez. The grapes were not in such good condition as the Bouschets. Being tender and juicy they suffered more in shipment. Wine yeast was found in this must in moderate numbers, but the wild yeasts were in such large numbers that the ratio between them was probably little different from that in No. 198.

No. 202 was second crop Zinfandel in very moldy condition.

No. 261 was Palomino must from grapes that arrived in very poor condition.

These observations show the necessity of crushing the grapes as soon as possible after gathering to prevent the growth of injurious molds. The practice of shipping grapes in boxes or loose in cars is objectionable as it gives the molds an opportunity to develop and depreciate the value of the grapes before they reach the winery. A much better practice is to crush the grapes at or near the vineyard into tank cars in which they are transported to the winery.

They show also the need of using sulfurous acid as soon as the grapes are crushed in order to prevent the development of the apiculate and other injurious yeasts which are always present. Grapes which are crushed and sulfited at the vineyard may be transported in tank cars in perfect condition to the winery even though they are two or three days on the way.

The need of using a starter of good yeast is also strongly indicated. This starter should be added at least several hours after the sulfiting. When sulfited grapes are shipped the yeast should be added as soon as the grapes are placed in the fermentation vats in the winery.

(*c*) *Methods of Transmitting Pure Yeasts.* The station has been supplying yeast cultures to wine-makers for many years and their utility has been thoroughly demonstrated. It is very important, however, that only a suitable yeast should be used. Some of the yeasts, sent out at first, were unsuited to Californian musts and conditions. As a rule, yeast is needed which is able to ferment musts of high sugar contents and which can withstand high temperatures. Certain German wine-yeasts tried failed in this respect. Many yeasts have given good results, but the most generally successful are those of Champagne and Burgundy type. These yeasts are thoroughly suited to our musts, and moreover have the excellent property of forming a heavy granular sediment which settles rapidly and leaves the wine bright very shortly after fermentation.

Occasionally yeasts sent to wineries have failed to develop. This is due to the fact that they soon lose activity in the liquid form in which

we have sent them and, unless used within a week from the time at which we prepare them, they are difficult to revive by the methods available to the wine-maker.

Such yeasts are not permanently injured and when revived by suitable means regain all their qualities. By transferring to fresh sterilized must and aerating thoroughly, they may in a few days be rendered active again. If placed in sterilized must without aeration, they may not develop at all. If put directly into fresh unsterilized must they may develop so slowly as to be overwhelmed by the wild yeasts present.

When yeasts are sent out to the winery to be used directly in fermenting vats as they are received they should not be more than a few days old when used. When used first to start a yeast-propagating apparatus, they may be used safely when they are several weeks old, providing proper precautions are taken to avoid contamination with other micro-organisms before they have regained their full activity. It has been shown that yeast grown on a solid culture medium retains its vigor much longer than when grown in a liquid medium. This is usually ascribed to the fuller aeration which the yeast receives when growing on the surface of a solid medium than when growing submerged in a liquid. It is well known that growing yeast with a full supply of air will increase its vigor, but the retention of this vigor is in this case more probably due to the fact that when growing on the surface of its nutrient medium it is protected to some extent from contact with its own products such as alcohol. Another factor is possibly that owing to the fixed position of the yeast and the solid condition of the medium, both the multiplication of the yeast and the exhaustion of the food supply are retarded.

Tests were made to compare the retention of vigor by yeast grown in each way. A series of tubes containing grape must was inoculated, half with yeast C and half with yeast B. A similar series containing solid agar must was inoculated, while warm enough to be fluid, in the same way. While the tubes of agar must were cooling they were rolled so that the solid nutrient material was spread in a thin layer over the inner surfaces of the tube. At intervals after inoculation, the contents of a tube of each lot were transferred to a flask of sterile must and, two days after this transfer, the number of living cells in the flask was counted. The numbers thus obtained give a measure of the number of living cells in the original tube at the time of the transfer, and of the rapidity with which they multiplied in the fresh must.

TABLE No. 31.

Relative stability of liquid and solid yeast cultures.

Age of culture in tubes.	Yeast C. Cells per cubic centimeter.		Yeast B. Cells per cubic centimeter.	
	Liquid culture.	Solid culture.	Liquid culture.	Solid culture.
1 day	105,000,000	40,000,000	170,000,000	80,000,000
2 days	65,000,000	85,000,000	105,000,000	105,000,000
3 days	92,000,000	95,000,000	100,000,000	125,000,000
4 days	45,000,000	65,000,000	93,000,000	110,000,000
16 days	50,000,000	25,000,000	28,000,000	71,000,000
32 days	4,000,000	13,000,000	7,500,000	43,000,000

These results indicate that during the first twenty-four hours the yeast is more efficient if grown in liquid medium. For a week or perhaps two there is little difference, but, at the end of a month, the advantage is very plainly with the yeast on solid culture medium.

Utilizing this property of solid cultures, a company in France prepares a form of yeast for wineries under the proprietary name of "Gelolevures." The yeast is grown on a thin layer of nutrient gelatine spread on a piece of thin cloth. Several of these pieces are suspended on a support inside a sealed tin case. The directions accompanying these tins are to remove the stopper, fill with sterilized must and place in a warm place. As soon as fermentation commences, the cover is removed and the whole contents of the tin are poured into a fermenting vat just filled with grapes. Tins of various sizes are supplied for vats of various capacities. The claim made for these yeasts is that they retain their vigor indefinitely and obviate the necessity of preparing a starter.

Sample tins of "Gelolevures" were received at the station for examination. To test their vigor, one of the tins, three months old, was treated according to the directions. At the same time a large flask, containing a liquid culture of yeast two months old, was used for comparison. The liquid in the latter was poured off the yeast sediment and the flask filled with the same must used to fill the tin of "Gelolevures." The must in the tin started to ferment sooner than that in the flask and finished fermenting in a shorter time.

The promptness with which the fermentation started in the "Gelolevures" demonstrated the superiority of this method of preparing yeast where it is to be used directly in the vats without the intermediate preparation of a starter, and where there is any considerable interval between the preparation of the yeast and its use.

A similar test was made with yeasts prepared in the laboratory. Two small flasks, in which a Champagne yeast was grown on a solid medium, were kept in the laboratory for five months. At the end of

that time, sterile must was added to the flasks, and was in full fermentation within twenty hours. Liquid cultures of the same yeast kept for the same length of time require careful manipulation to revive them. The reviving of a weak culture cannot safely be undertaken at the winery, owing to the great chance of contamination from outside organisms. A strong, solid culture, on the other hand, such as those contained in these flasks or the "Gelolevures," could easily be used to make a starter by ordinary methods easily employed by a competent wine-maker.

These tests all indicate the superiority of solid cultures for transmitting yeast for use in wineries, and most of the cultures sent out by the station during the last vintage were in this form.

(*d*) *Methods of Use in Wineries.* 1. DIRECT APPLICATION. There are two general methods of using pure yeast in wineries. The simplest in theory is to obtain from a reliable pure yeast laboratory a culture of yeast for each vat of grapes or cask of must to be fermented. This method, however, is expensive, involving the buying and transportation of large quantities of yeast.

It has been found, empirically, that it requires about 1 per cent of yeast to properly start a vat of sulfited grapes or defecated must. This means that ten gallons of a vigorous culture of yeast should be added to 1,000 gallons of must to ensure that the fermentation is due principally to the yeast added.

Determinations of the numbers of cells present in yeast cultures, and in sulfited musts at the winery indicate that these proportions are suitable. An average vigorous culture of wine-yeast in grape must will contain about 200,000,000 active cells per cubic centimeter. A properly sulfited must will contain no injurious wild yeast, and the natural wine yeast will be reduced to a few tens of thousands per cubic centimeter, even in musts made from grapes in bad condition. A 1 per cent addition of a vigorous culture of pure yeast would introduce approximately 2,000,000 cells per cubic centimeter into the whole mass. The pure yeast cells, therefore, would be, even in unfavorable cases, a hundred times more numerous than the natural yeast cells, while, in ordinary musts made from clean grapes, promptly crushed and sulfited, a 1 per cent addition of pure yeast would cause the added yeast to outnumber the natural yeasts many thousand times, and insure a fermentation which would be practically pure so far as contamination with other yeasts is concerned.

A smaller addition than 1 per cent, however, is not found advisable in practice, except in very hot weather, when it may be reduced a little. If too little is used, the start of fermentation is unduly prolonged, and

more than seems theoretically necessary must be used as a safety factor to provide against possible unusual contamination of the grapes or lack of vigor of the culture.

To start the fermentation of a vat, containing 1,000 gallons of crushed grapes by adding a liquid yeast culture directly, would require 10 gallons, which, with the glass containers, would weigh approximately 150 pounds. If solid cultures were used, prepared in glass flasks or bottles, such as were sent out by the station during the last vintage, the weight could be reduced to about 50 pounds. The tin cases of the "Gelolevures" make it possible to still further reduce this weight to about 15 pounds. Even the last weight is considerable, and the amount of yeast necessary makes the method very expensive.

2. PREPARATION OF A STARTER. Several years of experience have thoroughly demonstrated that it is perfectly practicable for any intelligent cellar-man to commence with a small culture of pure yeast, and prepare his own yeast from this in the form of a starter in any quantity needed.

The method is perfectly simple and equally adapted to large or small cellars. Expensive equipment in the form of pure yeast propagators, such as are used in breweries, is unnecessary and, in fact, dangerous in untrained hands. All that is needed is an outfit of tubs, vats and casks, such as are found in every cellar. These will vary in number and size, according to the scale on which wine is made and the arrangement of the cellar. The yeast apparatus, which was used in our winery experiments during the last vintage, and which is described later, illustrates the principles of the method, and will be found perfectly practical for a small winery. Modifications to facilitate handling and to adapt it to larger operations can easily be devised.

(*e*) *Rejuvenation and Increase of the Pure Culture.* When the culture of pure yeast reaches the winery, it should be as fresh and vigorous as possible. The cellar-man must first determine whether it has the necessary vigor and rejuvenate it, if necessary.

The next step is to increase the amount of pure yeast until it is sufficient to inoculate the first starting tub or vat. The quantity necessary will depend on the size of the apparatus. Up to this point special precautions should be taken to avoid contamination of the culture, and to insure that the yeast used to inoculate the starting tub is quite pure.

The final step is the increase of the yeast in the starting tub until it is sufficient to start the first fermentations. The starting tub or tubs should be large enough to produce each day all the yeast necessary to start all the grapes crushed in one day. A new tub, or series of tubs,

therefore, must be prepared each day during the vintage. The rejuvenation and preliminary increase, however, are needed only for the first. All subsequent tubs are inoculated from the tub of the previous day. Details of the various operations are given below.

(*f*) *Directions for propagating yeast in wineries.*

Step I. *Rejuvenation.* (1) SOLID CULTURES. A culture of pure yeast is received from the station. This culture is contained in a quart bottle, the neck of which is closed with a tight plug of cotton. The interior of the bottle is coated with a layer of solid culture medium, consisting of agar-agar and grape must. In this medium and on its surface the yeast is growing.

1. Obtain one quart of must from clean, sound grapes not overripe and place in a one quart Mason preserving jar, which, with its cover, should be thoroughly sterilized with boiling water before use. The jar should not be filled to nearer than one inch from the top.

2. Place the jar of must in a pot containing enough water to reach about half way up the side of the jar and deep enough, so that it can be covered when containing the jar. Place its cover loosely on the jar, but do not screw down.

3. Cover the pot and place on stove until the water boils. Continue boiling for ten to fifteen minutes.

4. Take the pot from the stove without removing the cover and allow to cool nearly to the temperature of the room.

5. Moisten the cotton plug and the neck of the bottle containing the yeast culture with a little alcohol. Apply a lighted match. This will destroy any spores which have settled on the neck of the bottle.

6. Remove the cotton plug with a pair of pincers, previously sterilized by dipping in alcohol and flaming.

7. Pour the sterilized and cooled must from the Mason jar into the opened bottle containing culture. This is best done by means of a glass or metal funnel, previously sterilized by dipping in boiling water. Avoid all contact of the fingers or other unsterilized surfaces with the neck of the bottle, the inside of the funnel or with the must. The bottle should be filled about four fifths full. Care should be taken not to wet the inside of the neck of the bottle with the must.

8. Pass the cotton plug quickly through the flame of an alcohol lamp and replace in the neck of the bottle.

9. Place the bottle in a warm place until fermentation starts. The temperature of the place should be as near 80° F. as practicable. If it is below 65° F. fermentation will be slow in starting; if much above 90° F. the yeast may be injured. A fireless cooker, containing one or two bottles of water warmed to 95° F., makes a good incubator. A

bucket or box, lined with wood wool ("excelsior"), in which the yeast bottle and two bottles of warm water are buried, and the whole covered with a blanket, is also suitable.

A culture treated in this way should be in full fermentation within twenty-four hours.

(2) LIQUID CULTURES. The manipulation of a liquid culture is similar, but requires some special precautions, owing to the possibility that the yeast is somewhat enfeebled by prolonged immersion in the liquid. The numbers of the paragraphs following correspond to those of the paragraphs above.

1. The quart of must should be placed in a two quart Mason jar, filling it not more than half full. A half gallon demijohn, plugged with cotton wool, and with the wicker cover removed, is even better.

2. Pour the contents of the *bottle* of pure yeast into the sterilized and cooled must in the two quart Mason jar and screw down the top of the latter. If a demijohn is used, replace the cotton with a scalded cork. Shake the mixture thoroughly for five minutes to aerate it. *Loosen* the top of the jar, or replace the cotton plug of the demijohn. This aeration is very necessary if the liquid culture is at all old, and may have to be repeated several times.

Step 2. *Increase.* The first step in the process has provided us with 1 quart of young vigorous yeast. This may be used directly to inoculate the first starting tub, but it is safer to increase it a little first unless we are working on a very small scale. At least 5 per cent of pure yeast should be used to inoculate the starting tub. The following directions are for the case where the starting tubs are intended to supply 25 gallons of starter per day, which is sufficient for 2,500 gallons of fermenting grapes; that is, the contents of one medium-sized fermenting vat. The directions can easily be modified for larger quantities.

1. Place 1½ gallons of clean, fresh grape must of about 20 per cent Balling in a two gallon demijohn, from which the wicker cover has been removed, and which has been sterilized with boiling water, and plugged with a tight roll of absorbent cotton.

2. Heat demijohn of must to boiling for fifteen minutes in a covered boiler, containing a few inches of water.

3. Remove from fire and allow to cool.

4. Remove cotton plug carefully and pour the quart of rejuvenated yeast into the demijohn of must, using the same precautions to prevent contamination explained in Step 1.

5. Aerate the must in the demijohn by shaking three or four times at

intervals of three to four hours, and leave in a warm place until vigorous fermentation occurs.

Step 3. *Inoculation of Starting Tub.* We now have 1½ gallons of pure yeast, which is sufficient to inoculate 30 gallons of must in a starting tub.

1. About twenty-four hours before the end of Step 2 place about 40 gallons of must in a suitable vat or barrel. Add 2 ounces of potassium metabisulfite (5 ounces per 100 gallons, equivalent to about 175 milligrams of SO_2 per liter), and allow to settle for about twenty-four hours. If the must is cold and made from clean, fresh grapes, the amount of sulfite may be reduced one third to one half with advantage.

2. Draw off 30 gallons of this clear, defecated must in to the starting tub and warm to 90° F. with boiling water. If the must is very sweet, the boiling water may be added to it directly. Six gallons of boiling water will raise 24 gallons of must from 60° F. to 90° F. If the must has 24 per cent Balling, the water will reduce it to about 20 per cent Balling. If the must has no excess of sugar, it may be warmed by floating in it a metal bucket or tub of boiling water, or by boiling 5 or 6 gallons of the must.

3. Add the 1½ gallons of yeast obtained by Step 2 to the warmed must in the starting tub.

4. Aerate thoroughly for ten minutes by dipping out bucketfuls of must and pouring back.

5. Cover tub with a canvas sheet to keep out dust and maintain the temperature. Aerate and warm two or three times, if necessary.

6. When the must in the starting tub is in full fermentation, it is ready to use for starting the regular fermentations.

(*g*) *Use of the Starter.* This preparation, which may seem somewhat complicated on paper, is really very simple when understood, and, moreover, is necessary only once. It should be done before the regular crushing of grapes commences, and the first tub of starter should be ready for the first load of grapes.

During the vintage, all that is necessary is to put one gallon of fermenting must from the starting tub into every one hundred gallons of crushed grapes. At the end of the day the fermenting must taken from the tub is replaced with fresh defecated must, and so on until the end of the vintage.

At least 10 per cent of the fermenting must should be left in the tub to start the new must added. If this is done, the starter remains practically pure until the end of the vintage. If the vintage is long, it might be advisable to add a fresh supply of pure yeast, developed from a new pure culture, to the starting tub about the middle of the vintage.

Where the yeast is needed only once a day, a single starting tub is all that is necessary; the size of the tub depending on the amount of yeast needed. Where the yeast is needed all day, it is necessary to have two tubs to be used on alternate days, as the must added at night will not be ready to use the next morning.

(*h*) *Stage of Maximum Efficiency of a Starter.* When we make a new addition of must to a starting tub, we dilute the yeast, so that there is a comparatively small number present in a given volume. The yeast immediately commences to multiply and finally reaches a maximum. It remains at this maximum number for some time, but the yeast cells gradually diminish in vigor.

If we use the starter too soon, therefore, we fail to obtain sufficient yeast cells to give us the full effect; if we use it too late, the yeast cells have lost some of their vigor, they are less efficient in overcoming the competing wild organisms, and the fermentation is slower.

There is theoretically some point between the commencement of fermentation and the end, at which the yeast in the starter has its maximum effectiveness. An experiment was undertaken to determine this point of highest efficiency and to discover some simple means of detecting it.

It is possible to determine the number of yeast cells present by means of a microscopical counting apparatus, but this gives us no measure of the vitality or degree of vigor of these cells. Some of the cells may be young and vigorous, some old and decrepit, some dead. By means of cultures on gelatine plates, a better estimate of the numbers of vigorous cells could be made, but this is troublesome and requires several days. Neither method is suitable for cellar use in the press of the vintage.

The age of the starter gives us a very uncertain measure. The condition of the yeast at the end of a certain number of hours will depend on many conditions, principally the temperature, the number and activity of the cells at the beginning, and the amount of aeration.

A simple guide was found in the disappearance of the sugar. As the development of the yeast progresses, the Balling per cent decreases. A certain decrease of Balling per cent indicates a certain amount of work done by the yeast, and is a measure of the number and vigor of the cells present. It remained to determine at what stage in the decrease of the Balling per cent the yeast possessed its highest efficiency as a starter. The following tests throw light on this matter.

A flask (A) containing must of 22.38 Balling and .75 per cent acidity was inoculated with a non-agglomerating wine yeast. The culture in this flask was used as a starter and a series of flasks, B to L, containing

the same must was inoculated each at different ages of the starter. The Balling per cent of the starter (flask A) was determined at each inoculation and the number of yeast cells present. By observing the course of fermentation in the various flasks we are able to determine what Balling degree of the must in A corresponds to the maximum efficiency of the yeast when used as a starter.

TABLE No. 32.

Correspondence of Balling per cent and number of yeast cells.
In Flask A (used as a starter for flasks B to L).

Hours.	Balling per cent.	Yeast cells per cubic centimeter.	1 per cent of starter added to
0	22.38		
18	20.80	5,620,000	Flask B
29	18.69	74,385,000	Flask C
43.5	13.10	122,322,000	Flask D
51.5	11.31	166,540,000	Flask E
66.5	7.58	175,631,000	Flask F
74.5	4.50	187,926,000	Flask G
80.5	.13	188,462,000	Flask H
88.5	0	190,000,000	Flask I
105.5	0	191,000,000	Flask J
113.5	0	189,000,000	Flask K
124.5	0	188,000,000	Flask L

Under the conditions of the experiments the number of yeast cells increased very rapidly until about half of the sugar had disappeared. After this it increased slowly until all the sugar was gone. Later it remained practically stationary until the end of the test. The comparative effectiveness of the yeast as a starter at the various stages is shown by the records of the other flasks.

TABLE No. 33.

Fermentation with starters of various Balling per cents and ages.

Flask B. Balling per cent of starter 20.8.			Flask C. Balling per cent of starter 18.69.		
Hours.	Balling.	Cells per cubic centimeter.	Hours.	Balling.	Cells per cubic centimeter.
0	22.38		0	22.38	
10.5	21.7	6,612,000	14.5	21.83	18,678,900
25.0	19.73	87,609,000	22.5	19.10	27,357,150
33.0	16.33	107,852,500	37.5	15.50	144,635,700
48	13.26	173,565,000	44.5	11.91	150,500,000
56	9.75	181,800,000	59.5	8.53	178,060,750
72	6.7	181,830,000	67.5	6.46	180,134,000
80	3.62		84.5	3.20	181,830,000
97	1.03	185,951,500	93.5	1.03	
106	0		108.5	0	183,635,000

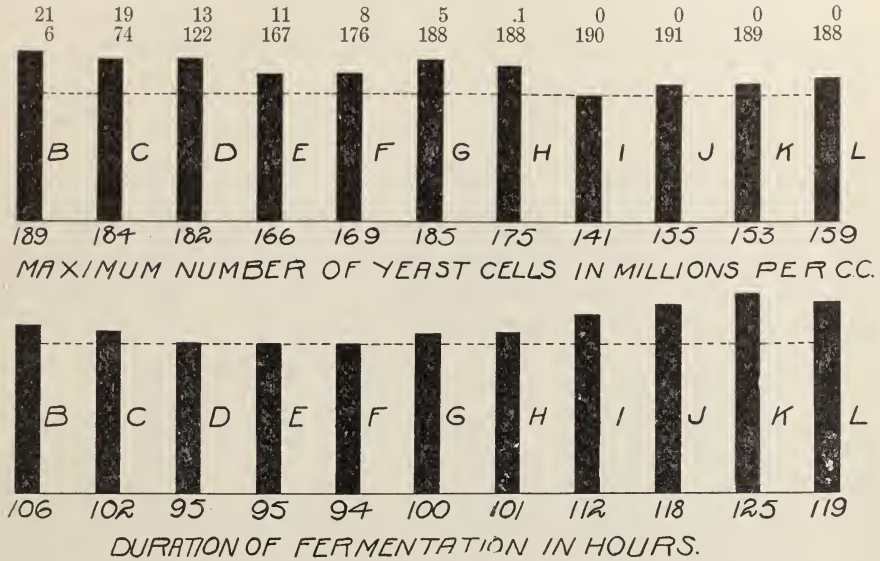
TABLE No. 33—CONTINUED.

Fermentation with starters of various Balling per cents and ages.

Flask D. Balling per cent of starter 13.1.			Flask E. Balling per cent of starter 11.31.		
Hours.	Balling.	Cells per cubic centimeter.	Hours.	Balling.	Cells per cubic centimeter.
0	22.38	940,000	0	22.38	
8	21.83	4,264,740	15	21.59	13,214,000
23	20.36	89,675,250	23	20.26	64,053,750
31	16.83	95,047,500	39	15.55	109,093,000
47	12.95	168,606,000	47	15.03	136,372,500
55	10.35	172,324,000	63	8.27	144,637,500
72	6.20	178,937,250	72	5.94	156,627,300
81	3.87		86	2.07	157,035,000
95	0	181,576,300	94	0	165,300,000
Flask F. Balling per cent of starter 7.58.			**Flask G. Balling per cent of starter 4.5.**		
0	22.38	144,634	0	22.38	
8	21.57	3,925,875	17	22.36	15,124,950
24	18.16	55,615,250	25	20.00	60,334,500
32	16.6	88,435,500	42	14.77	92,154,750
50	11.91	120,975,000	51	12.17	100,923,750
59	8.79	133,066,500	65	7.63	103,312,500
73	4.65	140,505,000	73	4.65	133,066,500
81	1.55	150,325,000	89	.1	180,177,000
97	0	169,432,500	101	— 0	183,483,000
Flask H. Balling per cent of starter .13.			**Flask I. Balling per cent of starter 0.**		
0	22.38		0	22.38	
8	21.83	2,727,400	17	22.09	14,050,500
25	20.00	57,615,250	26	19.73	56,202,000
33	16.86	78,517,500	40	16.1	78,517,500
47	12.43	107,445,000	49	11.91	99,180,000
55	8.79	138,851,000	65	8.27	115,710,000
71	4.78	140,505,000	77	7.24	134,719,500
83	2.3	152,076,000	95	2.3	135,546,000
101	0	175,218,000	113	0	140,505,000
Flask J. Inoculated 18 hours after starter reached 0.			**Flask K. Inoculated 26 hours after starter reached 0° Balling.**		
0	22.38		0	22.38	
33	22.09	5,372,250	8	21.31	7,438,500
47	17.7	28,927,500	24	20.78	32,233,500
55	17,38	55,788,750	32	19.34	85,129,500
71	13.47	95,874,000	54	13.21	96,600,500
83	10.87	113,230,500	72	8.27	117,189,500
98	6.72	129,760,500	95	3.6	142,984,500
116	2.32	144,464,000	125	0	152,902,500
139	0	154,555,500			
Flask L. Starter used 36 hours after fermentation was over in starting flask.					
0	22.38				
8	21.83	5,785,500			
28	20.78	64,467,000			
46	13.47	108,271,500			
69	6.9	133,066,500			
99	.5	159,514,500			
117	0	158,614,300			

DIAGRAM 7.

Influence of stage of starter (indicated by Balling °) on subsequent fermentation.



Stage of starter (A) expressed by Balling ° and millions of yeast cells per cubic centimeter (see Table No. 32).

The data of Tables 32 and 33 are brought together in Diagram 7. The fermentation in flasks D, E and F in which the starter was used when the Balling per cent had fallen to 13, 11 and 8 per cent respectively, was the most rapid. In flasks B and C in which the starter was used soon after commencing to ferment the fermentation required about twelve hours more. This was probably due to the fact that the starter contained less yeast and more time was required for the yeast to increase sufficiently to finish the fermentation. In flasks I, J, K and L in which the starter was used after it had ceased to ferment the fermentation was still slower, requiring about twenty-four hours more. This is undoubtedly due to a weakness of the activity of the yeast as the number of yeast cells in the starter was at its maximum when used.

These results indicate that the starter reaches condition of maximum efficiency about the time that the Balling per cent has fallen to 10 per cent. This would probably vary with the Balling per cent of the original must used in making the starter and with other conditions, but as the conditions were in most respects similar to those under which a starter would be prepared in a winery, they justify the advice given to use the starter when about half of the sugar has disappeared.

As the starter must be used for several hours during the day, it would be impracticable to use it always at the point of maximum efficiency, and this is not necessary as it retains very nearly the maximum for some time. A sufficient rule for practice is to wait until 10 per cent of the Balling has disappeared and then use it as long as some sugar still remains.

The time required after the addition of new must to bring a starter to this condition depends principally on the amount of yeast left in the starting tub and on the temperature of the cellar.

If the starter develops too slowly, it can be accelerated by leaving a larger amount of yeast. This will usually control the time perfectly, except in the case where very small yeast tubs are used or in very cold weather, in which cases it may be necessary to warm the liquid. Too rapid development of the starter is to be controlled by the opposite measures. Where very large yeast vats are used, a cooling coil may be necessary.

Winery tests with the simple yeast apparatus described later showed that when a new addition of must was added to the yeast tub at 10 a. m. the yeast was ready for use by 6 a. m. the next morning. It is necessary, therefore, to have only one yeast tub if but a limited number of fermentations are to be started. If fermentations are to be started all day, it would be necessary to have two tubs—one for the morning and one for the afternoon.

The morning tub could be filled at about 10 a. m. and would be ready to use from 6 a. m. to 10 a. m. of the following day, and could be filled immediately with fresh must for the next day. The afternoon tub could be filled at 5 p. m. and would be ready for use from 1 to 5 p. m. of the following day. This would give twenty hours for the yeast to develop to maximum efficiency and four hours in which to use the yeast.

(*i*) *Simple Yeast Apparatus for Wineries.*—The simple apparatus used for our winery tests is suitable for any ordinary cellar and with modifications for large cellars.

For the preliminary operations of the rejuvenation and increase of the pure yeast the following articles are needed:

1. A one quart solid culture of pure yeast.
2. A one quart Mason jar with grape must.
3. A covered saucepan to hold the Mason jar.
4. A small alcohol lamp.
5. A pint of alhohol.
6. A glass funnel to fit into the neck of a quart bottle.
7. A 1 pound roll of surgeon's cotton.
8. Two quart bottles for hot water.
9. A box 16 in. by 16 in. and 24 in. in depth for use as an incubator.
10. Wood wool (excelsior) to fill the incubator.
11. A blanket to cover the incubator.
12. A 2 gallon demijohn without a cover.
13. A wash boiler to hold the 2 gallon demijohn.
14. Four small tubs or vats to hold 50 gallons each.
15. A small scale to weigh 5 pounds, sensitive to ¼ of an ounce.
16. A one pint conical graduate.
17. 5 pounds of potassium metabisulfite.
18. 4 two gallon demijohns to hold the sulfite solution made by dissolving 10 ounces of potassium metabisulfite in one gallon of pure water. One pint of this solution will contain 1¼ ounces of sulfite, which is exactly what is needed to defecate 25 gallons of must.

The incubator, Fig. 7, is a simple wooden box large enough to hold a two gallon demijohn with space for one or two inches of excelsior below, around and above the glass. If the must in the demijohn is

Fig. 6.—Apparatus for the rejuvenation of pure yeast.

warm when put in the box and the whole covered with a thick blanket, it will keep its heat perfectly in the coldest night. A quart bottle requires the aid of a couple of bottles of hot water. The water in these bottles must not be too hot nor the insulation too perfect or the heat evoked by the fermenting must may raise the temperature too high and injure the yeast.

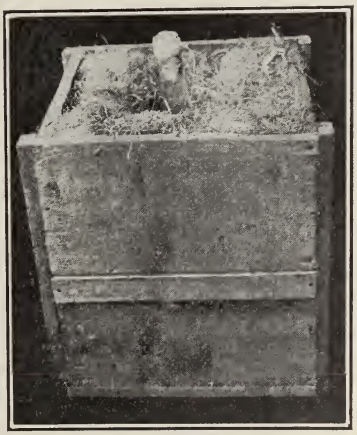


Fig. 7.—Incubator for pure yeast.

An ordinary wash boiler made for heating on a stove is too small to hold a two gallon demijohn upright but will do so if the demijohn is inclined. It is necessary to make some kind of frame to fit into the boiler and hold the demijohn. This frame can be made of wood or of wire. It should keep the demijohn from touching the bottom of the boiler to prevent the direct heat of the fire from cracking the glass. If the inclination of the demijohn is just sufficient to allow the cover of the boiler to be put in place, it will hold the needed $1\frac{1}{2}$ gallons of must.

Two of the fifty gallon vats are for the propagation of the yeast starter and two for the defecation of the necessary must. They should be furnished with faucets at the bottom for drawing off the must and yeast and with wooden or canvas covers to keep out dust. The defecating vats should be placed in such a position that the must can be run directly from them into the yeast vats. The arrangement adopted in our winery tests is shown in Fig. 8 and was found satisfactory.

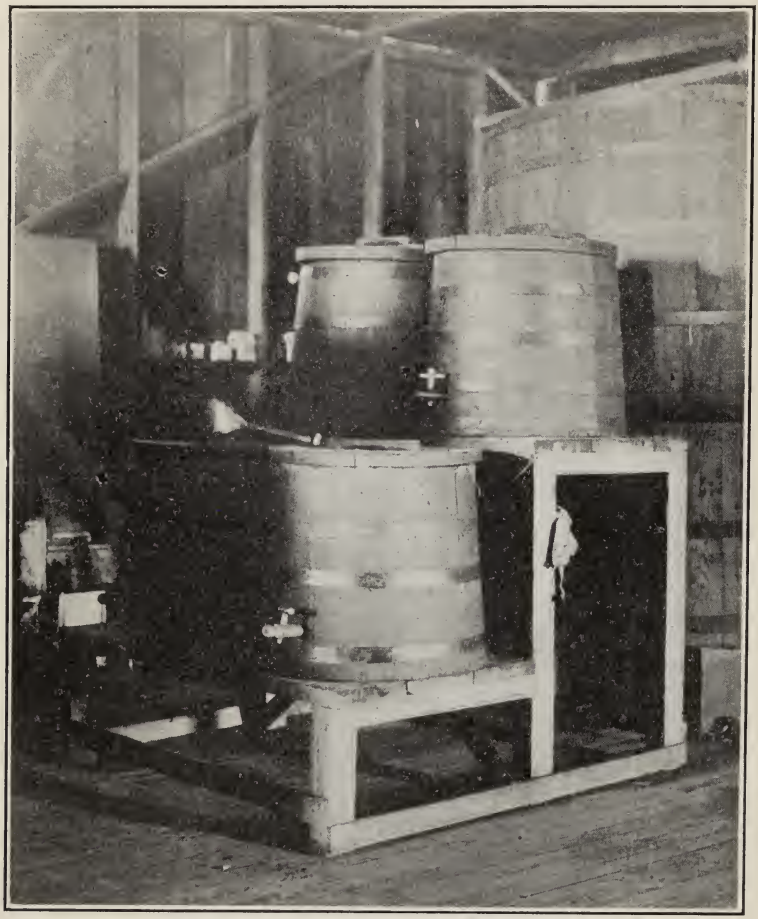


FIG. 8.—Yeast propagating apparatus for a winery.

The battery of vats should be so located that it is easy to supply them daily with must and to convey the yeast to the fermenting vats. It is convenient to label these defecating vats D_1 and D_2 and the corresponding yeast vats Y_1 and Y_2.

The first vat of starter Y_1 is prepared as described on pages 76 to 78. About 12 to 24 hours before the yeast in this vat is used, 30 gallons

of must should be placed in vat D_1 and sulfited with 1 pint of the sulfite solution. This must will be ready for vat Y_1 as soon as the yeast in this vat has been used. Before taking yeast out of the vat it should be well mixed in order to stir up the yeast which has settled. After 25 gallons of yeast have been used, 5 gallons remain for the inoculation of the new supply of must. Twenty-five gallons of must are then run directly from vat D_1 into vat Y_1. It is usually not necessary to warm the must, as the 5 gallons of vigorous young yeast left in the vat insure a prompt start of fermentation which will maintain a favorable temperature if the vat is kept covered. Aeration twice or three times during the day is advisable as it tends to increase the number and vigor of the yeast cells. Vats Y_2 and D_2 are treated in the same way. By properly timing the sulfiting in vats D_1 and D_2 and the transference of the defecated must from these vats to vats Y_1 and Y_2 a continuous supply of yeast can be obtained from 6 a. m. until 6 p. m. The following table will show how this can be done:

TABLE No. 34.

Times for filling and using defecating and yeast vats.

Time.	Vats.			
	Y_1.	D_1.	Y_2.	D_2.
September 1, 12 m.		fill		
September 1, 6 p. m.				fill
September 2, 12 m.	fill	fill		
September 2, 6 p. m.			fill	fill
September 3, 6 a. m.	use			
September 3, 12 m.	fill	fill	use	
September 3, 6 p. m.			fill	fill
September 4, 6 a. m.	use			
September 4, 12 m.	fill	fill	use	
September 4, 6 p. m.			fill	fill

Thus defecating vat D_1 is filled and sulfited at 12 m. every day. Twenty-four hours later at 12 m. of the following day the defecated must is transferred from this vat to yeast vat Y_1 and replaced with fresh sulfited must. Eighteen hours later at 6 a. m. of the third day the yeast in vat Y_1 is ready for use and can be employed until 12 m. when the vat must be filled again from vat D_1. The other pair of vats is treated in the same way except that they are filled at 6 p. m. and used from 12 m. to 6 p. m.

By this arrangement, the sulfited must always defecates for twenty-four hours and the yeast propagates for from eighteen to twenty-four hours, times which have been found suitable and sufficient. Any modifications of these times may be made which allow at least twelve hours for defecation and sixteen hours for fermentation. The defeca-

tion should not last more than forty-eight hours, and the yeast should be used before the must becomes quite dry.

A battery of the size described is sufficient to supply yeast for a winery fermenting 5000 gallons of wine per day. For smaller wineries two vats only might be used; for larger wineries the vats should be larger. A battery consisting of four 500 gallon vats would supply yeast for the fermentation of 50,000 gallons a day. Such a battery should be supplied with cooling coils, aerating device and with hose and small electric or other motor pump to fill the defecating vats and to transport the yeast to the fermentation tanks. One man could do all the work necessary in running the largest apparatus.

(*j*) *Precautions Necessary for the Proper Working of the Yeast Propagation.*

1. Cleanliness. Everything that comes in contact with the yeast should be kept clean. Buckets, tubs, hose and pumps used in handling the must and yeast should be washed every time they are used and immediately after using. Rinsing with a sulfite solution is useful but not necessary, if the utensils are placed where they will dry quickly after washing. The yeast vats should be kept covered both to keep out dust and to retain the heat.

2. Actions of the Sulfite. The must should not be transferred to the yeast vat until about twelve hours after sulfiting. This is to allow time for the sulfurous acid to enter into the combined form. The wild yeasts and other injurious micro-organisms are thus subjected to the action of the full amount of free SO_2 when it is added, while the culture wine yeast comes in contact only with a small amount of free SO_2 after the main part has entered into combination. The consequence is that the culture yeast retains its vigor and the wild organisms are paralyzed.

3. Maintenance of Vigor of Yeast. Fresh must should be added to the yeast vats sufficiently often to keep the fermentation going continuously. If the must in the yeast vats is allowed to become perfectly dry the yeast loses in vigor. When this occurs the yeast can be made vigorous again in two or three days by adding fresh must and thoroughly aerating.

4. Hastening Development. If the yeast develops too slowly, aerate more and if necessary warm.

5. Retarding Development. By leaving a smaller amount of yeast in the vat when adding fresh must, its development can be retarded. This may be done when a day or more passes without crushing as on

Sundays. The yeast left, however, should be at least 5 per cent of the volume of the must added. Less aeration will also tend to retard the growth of yeast.

6. Plan all operations so that the yeast can be used at its maximum efficiency, that is when the Balling per cent has fallen to between 12 per cent and 2 per cent.

IV. TESTS OF THE USE OF SO_2 AND PURE YEAST IN A WINERY.

(a) *Objects and Nature of the Tests.* Laboratory investigations and tests conducted in wineries with the coöperation of the owners have demonstrated thoroughly the utility of the use of pure yeast in combination with sulfurous acid and cooling machines. Many wine-makers, however, have an exaggerated idea of the cost and trouble involved in the use of devices for the control of temperature. The winery tests made this year had for their principal objects the determination of the possibility of introducing some of the benefits of pure yeasts with the help of sulfurous acid, and without any change in the usual methods of the winery involving the installation of cooling machinery or other new appliances except a simple yeast propagator.

Concurrently, observations were made to test on a larger scale certain conclusions reached by means of laboratory experiments.

The tests consisted of parallel series of fermentations, in one of which the ordinary methods of the winery were employed, and in the other, sulfurous acid and pure yeast separately or in combination were applied with as little change in the usual methods as possible. Observations were made on the progress of fermentation and on the development of the wine so long as it was possible to keep the various lots separate. The winery was peculiarly well adapted for the tests proposed. It is situated in a cool region where the need of temperature regulation is less pressing than in most parts of California; the winery is well supplied with the ordinary appliances and labor saving devices; and finally, it received grapes from both interior and coast regions, which enabled us to make our demonstrations on raw materials of differing character.

(b) *Changes in the kind and number of active micro-organisms.* Two vats, each holding 5 tons of grapes, were filled in the ordinary way with Zinfandel from Acampo. Experiment 171, vat 20, received the ordinary treatment of the cellar. Experiment 169, vat 4, was sulfited by the addition of three quarters of a pound of potassium metabi-

sulfite to the ton added gradually as the vat was filled. Five hours after the addition of the last sulfite, 50 gallons of pure yeast were stirred in.

At the start of perceptible fermentation the must in each vat was examined by means of plate cultures. *In the untreated vat nothing was found but molds and wild yeasts; in the treated vat nothing but wine yeast.*

Similar tests were made with two other vats with the following results:

TABLE No. 35.

Effect of SO_2 and pure yeast starter on micro-organisms.

Experiment 198. Vat 11. Zinfandel from Contra Costa County.

	Before sulfiting with 12 ounces per ton.	After adding yeast 1.5 per cent starter.
Molds	59,400 per c.c.	0
Wild yeasts	27,700	0
Wine yeasts	0	all

Experiment 202. Vat 19. Second crop Zinfandel from Acampo (very moldy).

	Sulfite 8 ounces per ton.		
	Before sulfiting.	After sulfiting.	After adding 1.5 per cent yeast.
Molds	1,600,000 per c.c.	0	0
Apiculate yeast	2,830,000	0	0
Other wild yeast	a few	0	0
Wine yeast	a few	560,000	2,816,000

Experiment 198 shows that in must from normal, sound, clean grapes, wine yeast exists in very minute quantities. The wild yeasts, (principally *apiculatus*) on the contrary, are numerous. Such a must allowed to ferment spontaneously without the addition of sulfurous acid or pure yeast would not develop wine yeast in appreciable quantities, until the apiculatus had multiplied sufficiently to seriously affect the quality of the wine. This is shown by experiment 202 where, owing to injury to the grapes and delay in crushing, the apiculatus yeast has increased a hundredfold. The first part of the fermentation of all our grapes is undoubtedly due to apiculatus when measures are not taken to prevent it. Much of the persistent cloudiness and delay in the finish of fermentation, even of otherwise good wines, is due to this cause.

The trouble with apiculatus fermentation is likely to be worse in the cleanest and best kept wineries and at the beginning of the season. When crushers, vats and other surfaces, with which the grapes come in contact are kept constantly covered with grape must the wine yeast

finally develops and inoculates the must. This is a defective method of obtaining wine yeast, however, as it gives no assurance of the kind of yeast and introduces also injurious bacteria.

The mold spores, while even in good grapes as numerous as the wild yeasts, are innocuous if the grapes are crushed while still in good condition as they do not increase appreciably in the must or crushed grapes. If delay occurs in the crushing, however, they may increase rapidly as shown by experiment 202, in which case they may seriously injure the wine.

In all three cases the injurious molds and yeasts were eliminated or paralyzed completely, even in vat 19 containing grapes in very bad condition. The number of wine yeast cells was so much smaller than that of the wild forms that before sulfiting it was difficult to find any at all. After sulfiting, however, in vat 19 they were found to exist in fair numbers. Sulfiting alone, therefore, would have insured a fermentation due principally to wine yeast in this case, though the molds and apiculatus yeasts had undoubtedly injured the must before the sulfite was added.

In vat 11 containing grapes in good condition, a starter of pure yeast insured a fermentation due practically entirely to the yeast added. In vat 19 the yeast to which the fermentation was due consisted of about four parts of that added to one part of the wine yeast occurring naturally on the grapes. This shows that even with grapes in very bad condition much improvement may be expected from sulfiting and pure yeast. The injurious yeasts are all eliminated and the slight admixture of the natural wine yeasts can do no harm.

These experiments show that the injurious activities of molds and wild yeasts can be prevented by prompt crushing and sulfiting followed by the addition of a starter of good wine yeast.

(c) *Duration and Course of the Fermentation.* 1. FERMENTATION CURVES. When a cask of grape must is allowed to ferment spontaneously the fermentation is not uniform throughout its course. There is first of all a period during which no fermentation is perceptible. This is because the yeasts present are insufficient to cause any noticeable changes in the mass. During this period the yeasts and other organisms increase in number until finally the mass exhibits signs of fermentation. These signs are rise of temperature, evolution of gas and decrease of sugar as shown by the Balling saccharometer. The yeasts continue to increase and the fermentation, at first slow, becomes more and more rapid until it reaches a maximum. After this the fermentation gradually becomes slower until it ceases completely. If it ceases before all the sugar has been destroyed it is said to "stick."

The character of the fermentation can be shown graphically by a

line or curve indicating the rate of fermentation at all points of its course. Curves of this kind can be made from observations of the volume of gas given off, of changes in the weight of the fermenting mass, of the rise of temperature or of the decrease of Balling per cent. The changes in the sugar contents as shown by the Balling saccharometer afford a very simple and useful means of forming a fermentation curve.

The shape of this curve shows the progress of the fermentation and the changes in its rapidity. The rapidity of the fermentation is influenced by a number of factors. It is the greater the larger the number of active yeast cells present and within certain limits the more fermentable matters contained in the must. It tends to decrease as the products of fermentation, alcohol, volatile acids, etc., accumulate in the must. Aeration tends to hasten the fermentation by promoting the multiplication of the yeast. High temperatures, within certain limits, accelerate the rate of fermentation by increasing the number of yeast cells and rendering them more active.

A typical fermentation curve is shown in Diagram 8. This represents a fermentation of sterilized must started with 1 per cent of vigorous yeast and kept at a nearly constant temperature.

Three periods may be distinguished, a fore period of two days in which the fermentation is slow, removing an average of 2° Balling per day; a central period of four days in which the fermentation is nearly twice as rapid, removing an average of $3\frac{3}{4}$° Balling per day; and an after period of $3\frac{1}{2}$ days in which fermentation becomes slow again, removing an average of only $1\frac{1}{3}$° Balling per day. If the temperature had been lower or the amount of yeast added less, the whole fermentation and especially the fore period would have been longer. If more aeration had been given the whole fermentation and especially the central period would have been shorter.

Curves of the fermentations that occur in practice in the winery show many interesting and instructive variations of this type.

The part showing the fore period is usually much longer and flatter. This is because the amount of yeast present in the freshly crushed grapes is small and the temperature low. It may be curtailed by the addition of a starter or by warming the must. It may be prolonged by the use of sulfites. Occasionally there is an actual rise of the curve during the fore period. This is due to the sugar in dried grapes, which diffuses into the must before noticeable fermentation commences.

The part showing the central period is usually shorter and steeper, particularly in red wine fermentations. This is because of greater aeration and a rise in temperature. It may be prolonged slightly by the use of cooling devices.

The part showing the after period often exhibits great variations. In very sweet musts it may be very much prolonged, may even last for months, and in some cases may become horizontal, showing a cessation of fermentation before 0 Balling° is reached. This is due to the accumulation of products of fermentation such as alcohol and volatile acids, which interfere with the action of the yeast. Aeration and maintaining the temperature are the principal means of preventing the undue lengthening of this period. Where the fermentation is pure and the temperature is high, especially in weak musts, this period may be much curtailed.

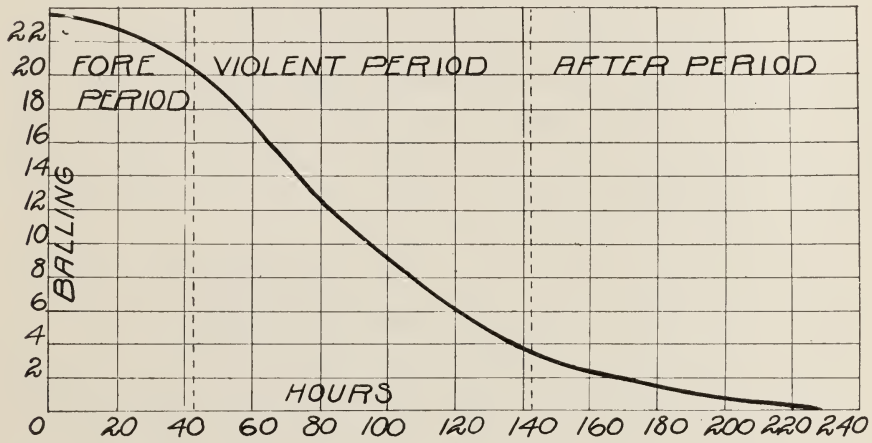


Diagram 8.

Typical fermentation curve at constant temperature.

2. RED WINE FERMENTATIONS. Observations were made on the following red wine fermentations at Martinez:

Variety and origin of grapes.	Sulfite per ton.	Pure yeast.	Fermenting vats.	Storage casks.
I. Zinfandel from Acampo	0	0	2	10
II. Zinfandel from Acampo	0	0	5	
III. Zinfandel from Acampo	5 oz.	plus	3	1
IV. Zinfandel from Acampo	12 oz.	plus	1	9
V. Zinfandel from Acampo	12 oz.	plus	4	
VI. Petite Sirah from Acampo	0	0	9	2
VII. Petite Sirah from Acampo	8 oz.	plus	7	16
VIII. Petite Sirah from Acampo	12 oz.	plus	10	
IX. Zinfandel from Contra Costa Co.	0	0	20	*10-11
X. Zinfandel from Contra Costa Co.	12 oz.	plus	8	12
XI. Zinfandel from Contra Costa Co.	12 oz.	plus	11	
XII. Alicante Bouschet from Acampo	8 oz.	plus	12	17
XIII. Alicante Bouschet from Acampo	8 oz.	plus	15	
XIV. Petite Sirah and Green Hungarian	9 oz.	0	6	18
XV. Barbera from Contra Costa Co.	5 oz.	0	14	4
XVI. Zinfandel, 2d crop from Acampo	8 oz.	plus	19	2
XVII. Alicante Bouschet from Acampo	8 oz.	0	13	26
XVIII. Zinfandel and Alicante Bouschet from Contra Costa Co.	6.5 oz.	plus	16	
XIX. Alicante Bouschet from Contra Costa Co.	8 oz.	plus	17	
XX. Zinfandel from Contra Costa Co.	8 oz.	plus	18	

*Puncheons.

The fermentations were all conducted in vats with 5-foot staves, holding about 2,200 gallons. No cooling was used, and, as a consequence, the temperatures in all cases were high. The temperature conditions of the locality and of the season were exceptionally favorable. The cellar varied on the average from about 61° F. at night to about 74° F., in the hottest part of the day. On the hottest day it rose to 84° F., and on the coldest night it fell to 54° F. Under less favorable conditions there would undoubtedly have been more trouble with "stuck" wines. The variations in the temperature of the air of the fermentation room during the vintage are shown by the curves of diagram 9.

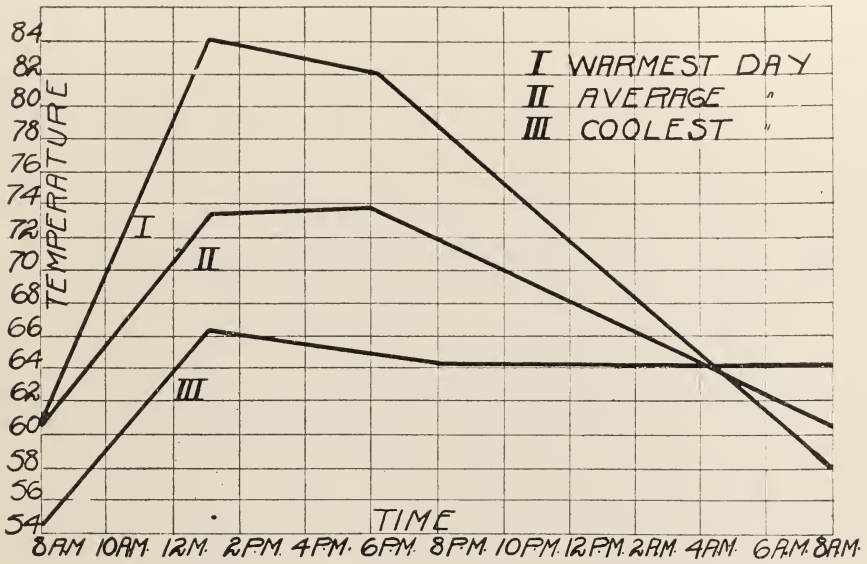


Diagram 9.

Variation in temperature of fermenting room.

The grapes, after stemming and crushing, were conveyed by means of a must pump directly to the fermenting vats. The sulfurous acid was added in fractional doses gradually as the vats were filled and mixed by repeated stirring. The sulfurous acid was used in the form of a strong water solution of potassium metabisulfite. After allowing the sulfited grapes to stand for some hours, 30 to 40 gallons of yeast were added. This represents a starter of from $1\frac{1}{2}$ to 2 per cent. The yeast was mixed as thoroughly as possible by means of punching. A better method would have been to pump over the must, as soon as the vat was filled, to distribute the sulfite equally and again while adding the yeast.

The vats were kept covered until the temperature began to rise, when the covers were removed and punching practised three times a day. When the temperature commenced to fall, the covers were replaced unless the vats were drawn off immediately. In most cases the drawing off was delayed until the Balling had fallen to 0°. A record was kept of the disappearance of the sugar and of the changes in temperature.

The first series of fermentations was made with Zinfandel grapes from Acampo, San Joaquin County. The grapes were in fair to poor condition, the different lots containing a smaller or larger proportion of dried and moldy grapes. Two vats were allowed to ferment in the ordinary way without sulfite or starter. One vat was sulfited at the

rate of 5 ounces to the ton, and two at the rate of 12 ounces. All three sulfited vats were started with pure yeast.

TABLE No. 36.

Fermentation I. Zinfandel from Acampo, Vat No. 2, ordinary fermentation, no sulfite, no starter.

Date.	Hour.	Balling per cent.	Temperature.			Remarks.
			Cap.	Bottom.	Room.	
Oct. 24	8 a. m.	23.6	--------	59.0	54.5	Started to fill vat. Grapes show many raisins.
Oct. 24	2 p. m.	23.7	--------	59.0	69.0	Vat half full. Grapes a little cleaner than in Vat 1.
Oct. 25	7 a. m.	24.1	--------	57.0	54.5	Slight signs of fermentation
Oct. 25	8 p. m.	24.6	--------	60.0	64.5	Slow fermentation. Smell of CO_2 gas.
Oct. 26	8 a. m.	24.8	--------	57.0	61.0	Slow fermentation. Smell of CO_2 gas.
Oct. 26	9 p. m.	24.2	--------	59.0	--------	Gas coming off freely.
Oct. 27	8 a. m.	24.1	--------	59.0	--------	Fermentation vigorous.
Oct. 27	9 a. m.	--------	--------	--------	--------	Started to complete filling of vat.
Oct. 27	2:30 p. m.	--------	--------	--------	--------	Vat full.
Oct. 27	8 p. m.	22.7	64.5	73.5	61.0	Fermentation of the grapes first crushed has raised the temperature of the bottom
Oct. 28	8 a. m.	22.1	70	66.0	59.0	
Oct. 28	12 m.	20.5	70	69.0	71.5	Temperature taken after punching.
Oct. 28	8 p. m.	16.0	80	78.0	70.5	Temperature taken after punching.
Oct. 29	8 a. m.	5.5	97	91.5	59.0	
Oct. 29	11 a. m.	2.7	99.5	--------	71.5	
Oct. 29	4 p. m.	1.6	100.5	100.5	72.5	Fermentation subsiding.
Oct. 29	10:30 p. m.	0	--------	97.0	--------	Drew off free run into cask 10.

The rise of Balling per cent from 23.6 to 24.8 during the first forty-eight hours is due to the presence of numerous dried grapes. It is not considered good practice to spread the filling of a vat over two or three days, as in this case, although it is said to promote the dissipation of some of the heat of fermentation where cooling devices are not employed. This tendency is very slight, however, as the temperature in fermentation II only rose about two degrees higher than in I, although the original temperature of the grapes was about 12 degrees higher.

TABLE No. 37.

Fermentation II. Zinfandel from Acampo, Vat No. 5, ordinary fermentation, no sulfite, no pure yeast.

Date.	Hour.	Balling per cent.	Temperature.			Remarks.
			Cap.	Bottom.	Room.	
Oct. 29	10 a. m.	----------	----------	----------	----------	Began filling vat.
Oct. 29	6 p. m.	----------	----------	----------	----------	Vat filled and covered.
Oct. 29	8 p. m.	21.7	----------	71.5	70.5	Slight signs of fermentation.
Oct. 30	8 a. m.	22.0	----------	64.5	61.5	
Oct. 30	8 p. m.	22.5	----------	64.5	71.5	
Nov. 1	8 a. m.	23.0	68.0	69.0	62.5	Slow fermentation.
Nov. 1	12 m.	22.5	68.0	69.0	71.5	Slow fermentation.
Nov. 1	8 p. m.	22.0	----------	----------	64.5	Fermentation well started.
Nov. 2	9 a. m.	17.0	79.0	77.0	59.0	Fermentation vigorous.
Nov. 2	12 m.	12.0	84.0	82.5	68.0	
Nov. 2	10 p. m.	4.0	100.5	99.5	68.0	
Nov. 3	8 a. m.	1.0	102.0	101.5	59.0	
Nov. 3	8 p. m.	.3	100.5	100.5	57.0	Fermentation slackening.
Nov. 4	8 a. m.	.2	97.0	97.0	61.0	Drew off free run into cask 10.

This vat also shows a rise in Balling in the first forty-eight hours, due to the presence of dried grapes.

TABLE No. 38.

Fermentation III. Zinfandel from Acampo, Vat No. 3, sulfite 5 ounces per ton, pure yeast.

Date.	Hour.	Balling per cent.	Temperature.			Remarks.
			Cap.	Bottom.	Room.	
Oct. 27	2 p. m.	----------	----------	----------	----------	Started filling vat.
Oct. 27	3 p. m.	----------	----------	----------	----------	Added 8 ounces sulfite.
Oct. 27	4 p. m.	----------	----------	----------	----------	Added 8 ounces sulfite.
Oct. 27	6 p. m.	----------	----------	----------	----------	Added 16 ounces sulfite.
Oct. 27	6:15 p. m.	----------	----------	----------	----------	Added 8 ounces sulfite.
Oct. 27	6:30 p. m.	----------	----------	----------	----------	Added 5 ounces sulfite.
Oct. 27	10:30 p. m.	----------	----------	67.0	61.0	Added starter of yeast.
Oct. 28	8 a. m.	22.3	----------	67.0	60.0	No visible fermentation.
Oct. 28	12 m.	22.3	----------	66.0	----------	Fermentation started very slightly.
Oct. 28	8 p. m.	22.3	66.0	66.0	71.0	
Oct. 29	8 a. m.	22.2	----------	68.0	61.0	
Oct. 29	12 m.	20.2	71.5	----------	70.0	Fermentation well started.
Oct. 29	8 p. m.	16.2	82.5	75.0	70.0	Fermentation vigorous.
Oct. 30	8 a. m.	14.9	82.5	----------	61.5	
Oct. 30	12 m.	11.5	88.0	86.0	76.0	
Oct. 30	6 p. m.	6.7	89.5	86.0	68.0	
Oct. 30	8 p. m.	5.0	97.0	97.0	73.5	
Nov. 1	8 a. m.	1.2	100.5	100.5	62.5	
Nov. 1	10:39 a. m.	.9	----------	100.5	64.5	
Nov. 1	4 p. m.	.3	----------	----------	----------	Drew off free run into cask 1.

TABLE No. 39.

Fermentation V. Zinfandel from Acampo, Vat. No. 4; sulfite, 12 ounces per ton; pure yeast.

Date.	Hour.	Balling per cent.	Temperature.			Remarks.
			Cap.	Bottom.	Room.	
Oct. 23	10 a. m.	21.9	--------	--------	--------	Started filling vat; grapes very moldy.
Oct. 23	10:30 a. m.	--------	--------	--------	--------	Added 8 ounces sulfite.
Oct. 23	11:30 a. m.	23.2	--------	64.5	--------	Added 24 ounces sulfite.
Oct. 23	2:30 a. m.	--------	--------	--------	--------	Added 32 ounces sulfite.
Oct. 23	6 p. m.	--------	--------	--------	--------	Added 40 ounces sulfite.
Oct. 24	9 a. m.	22.5	--------	64.5	54.5	
Oct. 24	9 p. m.	22.5	--------	64.5	64.5	No fermentation.
Oct. 25	7:30 a. m.	22.5	--------	62.5	54.5	A few bubbles of CO_2.
Oct. 25	8:30 a. m.	--------	--------	--------	--------	Added 15 ounces sulfite.
Oct. 25	11:30 a. m.	22.5	--------	--------	66.0	Added 50 gallons pure yeast.
Oct. 25	8 p. m.	23.5	--------	68.0	64.0	Slight fermentation.
Oct. 26	1 p. m.	23.6	70.0	68.0	64.5	Fermenting slowly.
Oct. 26	9:45 p. m.	23	--------	68.0	61.0	Fermenting well.
Oct. 27	7:45 a. m.	22.7	--------	68.0	--------	
Oct. 27	11:40 a. m.	22.1	70.0	68.0	--------	
Oct. 27	8 p. m.	20.5	74.5	71.5	61.0	
Oct. 28	8 a. m.	16.3	95.0	82.5	60.0	Removed cover.
Oct. 28	12 m.	11.2	--------	84.0	71.5	
Oct. 28	8 p. m.	5.3	100.5	97.0	70.5	
Oct. 28	12 p. m.	2.5	100.5	98.5	--------	
Oct. 28	1 a. m.	--------	--------	--------	--------	Drew off into cask 9.
Oct. 28	2:30 a. m.	2.7	97.0	--------	--------	
Oct. 29	8 a. m.	1.8	--------	99.5	--------	
Oct. 29	11:30 a. m.	.7	--------	100.5	70.0	

This fermentation shows very clearly the ineffectiveness of SO_2 in controlling temperature. The temperature reached 97° F., while the must still showed over 5° Balling. The wine, however, continued to ferment and became dry. Without the sulfite it would probably have "stuck" and remained sweet.

TABLE No. 40.

Fermentation IV. Zinfandel from Acampo, Vat. No. 4; sulfite, 12 ounces per ton; pure yeast.

Date.	Hour.	Balling per cent.	Temperature.			Remarks.
			Cap.	Bottom.	Room.	
Oct. 28	8 a. m.	--------	--------	--------	--------	Commenced to fill vat.
Oct. 28	10:30 a. m.	--------	--------	--------	--------	Vat filled; 7½ pounds of sulfite added in all.
Oct. 29	3 p. m.	22.7	--------	--------	--------	Added 40 gallons pure yeast.
Oct. 30	8 a. m.	22.7	--------	66.0	61.5	Slight signs of fermentation.
Oct. 30	8 p. m.	22.3	71.5	64.5	71.5	
Nov. 1	8 a. m.	22	70.5	68.0	62.5	Vigorous fermentation.
Nov. 1	8 p. m.	20	68.0	68.0	64.5	
Nov. 2	7 a. m.	16	89.5	71.5	61.0	
Nov. 2	12 m.	12	84.0	82.5	--------	
Nov. 2	10 p. m.	3.5	98.5	97.0	71.5	
Nov. 3	8 a. m.	0	100.5	100.5	59.0	
Nov. 3	2 p. m.	--------	--------	--------	--------	Drew off free run into cask 9.

The foregoing tables and diagram 10, made from them, afford us a means of judging of the influence of sulfiting and starters on the course of the fermentations. A comparison of the five fermentations is made in Table 41.

TABLE No. 41.

Comparison of fermentations with and without SO_2 and pure yeast. Summary of Fermentations I, II, III, IV and V.

	Balling per cent.	Temp.	Sulfite.	Yeast.	Fermentation.			Sugar lost per 24 hours.		
					Commenced.	Lasted.	Total.	A From filling.	B From starting.	C From visible ferment.
I	24.8	59.0	0	0	52 hrs.	82 hrs.	134 hrs.	4.44	4.44	7.26
II	23.0	71.6	0	0	36 hrs.	72 hrs.	108 hrs.	5.11	5.11	6.67
III	22.3	67.1	5 oz.	4 hrs.	34 hrs.	58 hrs.	92 hrs.	5.82	6.08	9.23
IV	23.6	64.4	12 oz.	50 hrs.	67 hrs.	79 hrs.	146 hrs.	3.88	5.90	7.17
V	22.7	66.0	12 oz.	29 hrs.	50 hrs.	68 hrs.	118 hrs.	4.62	6.12	8.01

The third column from the end (A) shows the influence of the combination of sulfite and a starter on the total time of fermentation. During the 134 hours, which the fermentation of No. I lasted, an average of 4.44 per cent Balling was lost every twenty-four hours. No. II required less time, 5.11 per cent Balling disappearing in twenty-four hours. This difference was due to the higher temperature of the grapes of No. II when crushed.

In No. III, 5.82 per cent Balling disappeared in twenty-four hours, which is more than shown by either untreated vat. This shows that the delay due to a small dose of sulfite is less than the gain in time, due to the addition of yeast, if the starter is added soon after the sulfiting.

In No. IV fifty hours elapsed from the crushing of the grapes until the addition of the starter. The use of the starter did not compensate for this loss of time and only 3.88 per cent Balling disappeared in twenty-four hours. In No. V, twenty-nine hours were allowed to elapse between the sulfiting and the addition of the starter, and the sugar disappeared at the rate of 4.62 per cent Balling in twenty-four hours. This rate is intermediate between the two untreated vats, and is just about what might have been expected from the original temperature of the grapes, which is also intermediate between those of the untreated vats.

These results indicate that a delay of the fermentation of twenty-four hours, due to sulfiting, can be overcome by the use of a starter. As other results show that the starter is best used within six to twelve hours after sulfiting, the combination of sulfite and pure yeast gives a net gain in the time of fermentaion. This gain is due to the time

needed by the natural yeast of the grapes to multiply sufficiently to cause perceptible fermentation.

In cellars where the vats, crushers and conveyors are allowed to remain covered with grapes and must they may supply a natural starter. If this starter happens to be principally wine yeast, the consequent fermentation may finish sooner than that of a sulfited vat started with pure yeast. If the natural starter, however, as will usually be the case, contains a large proportion of injurious organisms the fermentation may be much longer, owing to delay in the latter part.

The second column from the end (B) shows the rate of disappearance of the sugar after the addition of the starter in III and IV and V. If we compare these figures with those of I and II in the third column from the end, we eliminate the effect of the sulfite, and have a measure of the effect of the starter alone. This indicates a considerable increase in the rate of fermentation. Basing the calculation on a comparison of No. IV with the mean of Nos. I and II, we have an increased rate of fermentation of about 23 per cent.

Finally, in the last column we have some light on the influence of the sulfite alone. The figures give the average loss of sugar per twenty-four hours from the start of visible fermentation to its completion at 0 per cent Balling. The most rapid loss is in No. III, which received a small dose of sulfite. No. II in spite of the higher temperature of the grapes, fermented at a rate about 17 per cent slower. This might indicate a superiority of the added pure yeast, but more probably indicates that in No. II the growth of wild yeasts diminished the activity of the natural wine yeast. Nos. IV and V, which received a larger dose of sulfite, fermented at about the same rate as Nos. I and II, which received none. This seems to indicate that the weakening effect in the yeasts of such doses of SO_2 was in this case about equal to that of the wild yeasts, so far as the activity of the fermentation is concerned. The effect on the quality of the wine, however, is quite different. The wild yeasts depreciate the quality of the wine, and may prevent the completion of the fermentation. No such effects have been noted to follow the use of these doses of SO_2.

A comparison of these five fermentations is made by the curves of diagram 10. The time of the addition of the pure yeast starter is shown at the right of the diagram. The curve beyond this time should be compared with the whole curve of the unsulfited vats. The whole work of the yeast is shown in this way to have required from $4\frac{1}{2}$ to 6 days in the unsulfited vats and from $3\frac{1}{2}$ to 4 days in the sulfited. The tumultuous fermentation, however, was more rapid in the unsulfited, as shown by the steeper curve representing this stage.

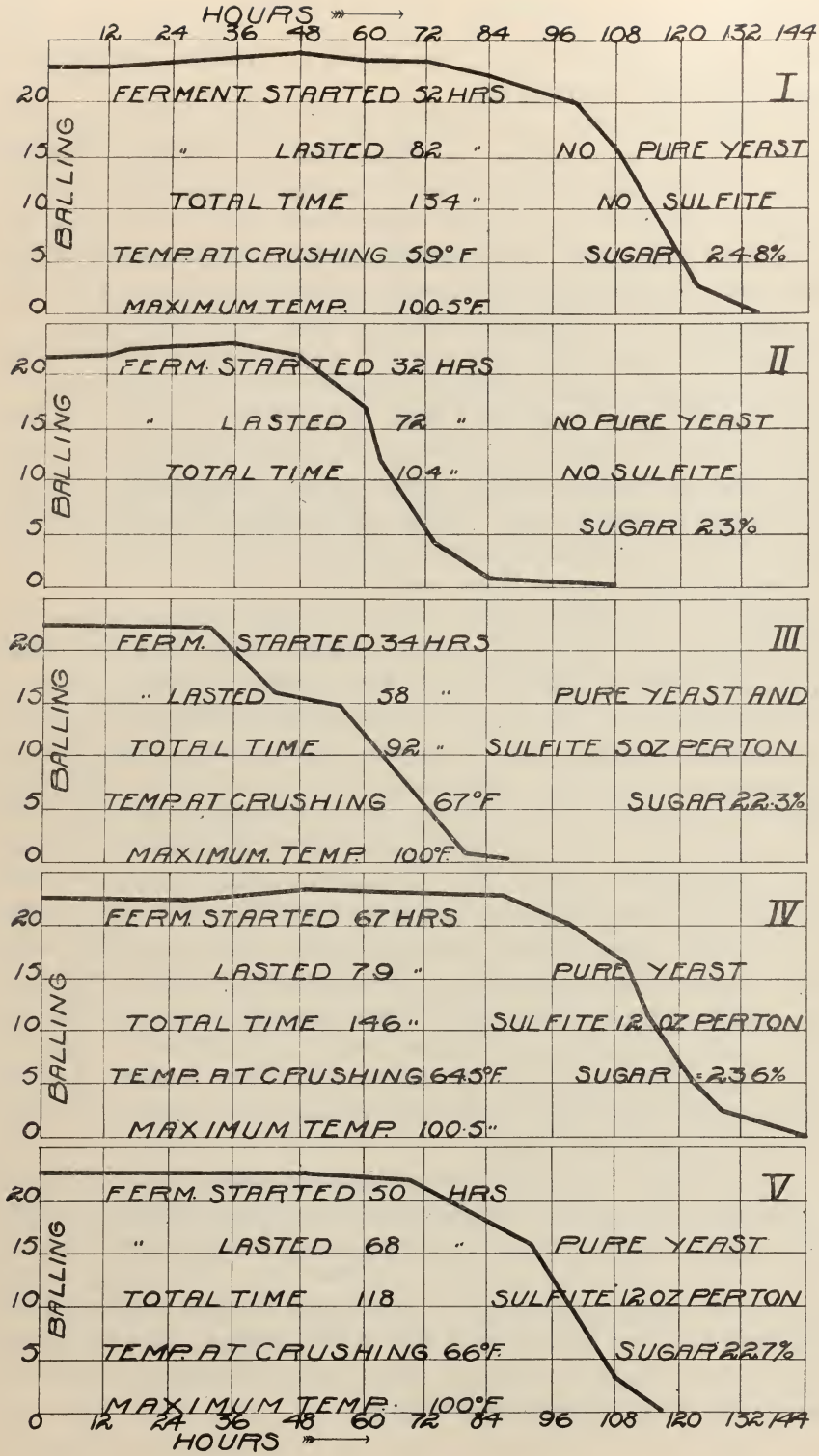


DIAGRAM 10.

Fermentation curves of Acampo Zinfandel.

TABLE No. 42.

Fermentation VI. Petite Sirah from Acampo, Vat No. 9, no sulfite, no starter.

Date.	Hour.	Balling per cent.	Temperature.			Remarks.
			Cap.	Bottom.	Room.	
Nov. 6	9 a. m.	--------	--------	--------	--------	Commenced to fill vat.
Nov. 6	6 p. m.	21.0	--------	72.5	72.5	
Nov. 7	8 a. m.	21.2	--------	61.5	60.0	
Nov. 7	1 p. m.	22.5	--------	62.5	71.5	
Nov. 7	6 p. m.	22.1	62.5	62.5	73.5	Fermenting slowly.
Nov. 8	8 a. m.	21.0	--------	65.5	58.0	
Nov. 8	1 p. m.	20.0	68.0	67.0	71.5	
Nov. 8	6 p. m.	20.8	68.0	68.0	71.5	
Nov. 9	8 p. m.	18.0	70.0	68.0	62.5	Fermenting vigorously.
Nov. 9	1 p. m.	16.0	80.5	80.5	66.0	
Nov. 9	6 p. m.	10.0	84.0	82.5	68.0	
Nov. 10	8 a. m.	4.5	--------	91.5	57.0	
Nov. 10	1 p. m.	1.5	100.5	98.5	70.0	
Nov. 10	5:30 p. m.	.5	100.5	100.5	--------	
Nov. 11	8 a. m.	0	95.0	97.0	--------	Drew off into cask 2.

TABLE No. 43.

Fermentation VII. Petite Sirah from Acampo, Vat No. 7; sulfite, 8 ounces per ton; pure yeast.

Date.	Hour.	Balling per cent.	Temperature.			Remarks.
			Cap.	Bottom.	Room.	
Nov. 4	1 p. m.	21.0	--------	--------	--------	Started to fill vat and added 4 pounds sulfite.
Nov. 4	5 p. m.	--------	--------	--------	--------	Finished filling gradually.
Nov. 5	9 a. m.	22.5	--------	59.0	61.0	
Nov. 5	1:30 p. m.	--------	--------	--------	63.5	Added 30 gallons of yeast.
Nov. 5	6 p. m.	22.4	--------	62.5	71.0	
Nov. 6	8 a. m.	--------	67	--------	60.0	Fermentation started.
Nov. 6	1 p. m.	21.0	86.0	64.5	70.0	Fermenting well.
Nov. 6	6 p. m.	20.0	--------	73.5	73.5	Fermentation vigorous.
Nov. 7	8 a. m.	13.0	96.0	88.5	58.0	
Nov. 7	1 p. m.	6.0	100.5	97.0	75.0	
Nov. 7	6 p. m.	1.75	100.5	97.0	71.5	
Nov. 8	8 a. m.	0	--------	98.5	70.0	
Nov. 8	9 a. m.	--------	--------	--------	--------	Drew off free run into cask 16.

TABLE No. 44.

Fermentation VIII. Petite Sirah from Acampo, Vat No. 10; sulfite, 12 ounces per ton; pure yeast.

Date.	Hour.	Balling per cent.	Temperature.			Remarks.
			Cap.	Bottom.	Room.	
Nov. 7	1:30 p. m.	--------	--------	--------	--------	Began filling vat and adding sulfite gradually.
Nov. 7	5:20 p. m.	--------	--------	--------	--------	
Nov. 8	8 a. m.	21	--------	66.0	58.0	Vat full.
Nov. 8	6 p. m.	21.75	--------	71.5	75.0	Added pure yeast.
Nov. 9	8 a. m.	21	--------	68.0	62.5	Fermentation starting.
Nov. 9	1 p. m.	16	--------	84.0	66.0	Fermentation vigorous.
Nov. 9	6 p. m.	10.5	89.5	83.5	68.0	
Nov. 10	8 a. m.	4	97.0	97.0	57.0	
Nov. 10	1 p. m.	1.5	102.0	98.5	70.0	
Nov. 10	5:30 p. m.	.25	102.0	98.5	68.0	Drew off into cask 16.

TABLE No. 45.

Summary of Fermentations VI, VII and VIII.

	Balling per cent.	Temp.	Sulfite.	Pure yeast.	Fermentation.			Sugar lost per 24 hours.		
					Commenced.	Lasted.	Total.	A From filling.	B From starter.	C From visible ferment.
VI -----	21	72.5	0	0	36 hrs.	74 hrs.	110 hrs.	4.58	4.58	6.80
VII -----	22.5	59	8 oz.	18 hrs.	25 hrs.	62 hrs.	87 hrs.	6.21	7.83	8.71
VIII -----	21.0	66	12 oz.	15 hrs.	25 hrs.	55 hrs.	80 hrs.	6.30	7.76	9.17

The results shown in Table 45 confirm the conclusions drawn from those of Table 41. Column A shows a net gain of over 35 per cent in the rapidity with which the sugar disappears, in favor of the vats receiving sulfite and pure yeast. This indicates, as in the previous experiments, that a delay of eighteen hours, due to sulfiting, is more than compensated for by the gain in time, due to the addition of a starter after that delay.

The gain, due to the starter alone, as indicated by column B, is about 70 per cent. Column C shows that the pure yeast fermented about 32 per cent faster in the sulfited must than the mixed natural yeasts did in the unsulfited.

The character of the three fermentations is shown graphically in diagram 11. Both the sulfited vats finished their fermentation before the witness, although the grapes were much colder when crushed. No. VIII finished before No. VII, because the grapes were warmer and not quite so sweet. There is no evidence that the larger amount of sulfite delayed the fermentation at all.

All three fermentations continued until the Balling saccharometer indicated 0. If the weather had been warm, it is very probable that the unsulfited vat would have failed to finish.

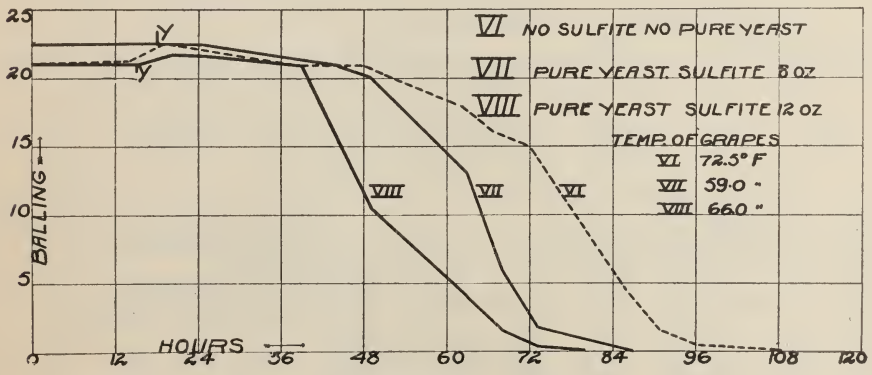


DIAGRAM 11.

Fermentation curves of Petite Sirah; VI, VII, VIII.

TABLE No. 46.

Fermentation IX. Zinfandel from Contra Costa County, Vat No. 20, ordinary fermentation, no sulfite, no starter.

Date.	Hour.	Balling per cent.	Temperature.			Remarks.
			Cap.	Bottom.	Room.	
Oct. 23	10 a. m.	24.5				Good condition; good many raisins (first load).
Oct. 24						Second load; reduced sugar.
Oct. 25						1½ loads; fermentation vigorous.
Oct. 25	6 p. m.	22.0		73.5	72.0	
Oct. 26	8 a. m.	19.0		75.0	60.0	
Oct. 26	11 a. m.	17.0	81	79.0	64.0	
Oct. 26	6 p. m.	14.5	86	84.0	65.0	
Oct. 27	8 a. m.	6.0	97	95.0	59.0	
Oct. 27	11:30 a. m.	4.5				Drew off 2 puncheons; rest of record refers to wine in puncheons.
Oct. 27	6 p. m.	3.5				
Oct. 28	6 p. m.	1.0				
Oct. 30	8 a. m.	.25				
Nov. 4		0				

TABLE No. 47.

Fermentation X. Zinfandel from Contra Costa County, Vat No. 8; sulfite 12 ounces; pure yeast.

Date.	Hour.	Balling per cent.	Temperature.			Remarks.
			Cap.	Bottom.	Room.	
Oct. 4	4 p. m.	24				First load crushed; added 2 pounds of sulfite.
Oct. 5	2 p. m.					Second load crushed; added 2 pounds of sulfite.
Oct. 5	5 p. m.					Third load crushed; added 1 pound of sulfite.
Oct. 6	10:30 p. m.	23.8				Fourth load crushed; added 2 pounds of sulfite.
Oct. 6	2 p. m.					Added 2 pounds sulfite.
Oct. 6	5:15 p. m.					Finished filling and added 2 pounds sulfite. Reduced sugar.
Oct. 6	6 p. m.	22.3		68	73	Added pure yeast.
Oct. 7	7:30 a. m.	22		70	60	
Oct. 7	6 p. m.	21		70	70	Fermenting.
Oct. 8	8 a. m.	19.3	80	73	58	
Oct. 8	1 p. m.	15		83	75	Very active.
Oct. 8	6 p. m.	11		86	72	
Oct. 9	8 a. m.	3		99	70	
Oct. 9	12 m.	2		100	66	
Oct. 9	5 p. m.	0	100	98.5	68	Drew off.

TABLE No. 48.

Fermentation XI. Zinfandel from Contra Costa County, Vat No. 11; sulfite, 12 ounces; pure yeast.

Date.	Hour.	Balling per cent.	Temperature.			Remarks.
			Cap.	Bottom.	Room.	
Oct. 9	10 a. m.					Began crushing; 2½ pounds sulfite added gradually.
Oct. 10						Continued crushing; three pounds sulfite added gradually.
Oct. 11						Continued crushing; two pounds sulfite added gradually.
Oct. 11	3:30 a. m.					Finished crushing; many raisins.
Oct. 11	6 p. m.	23.5				Reduced sugar and added pure yeast.
Oct. 12	8 a. m.	22		64	61	
Oct. 12	1 p. m.	21.8		70	67	Fermentation started.
Oct. 12	6 p. m.	20.5		69	74	
Oct. 13	8 a. m.	18	75	74	61	Fermenting actively.
Oct. 13	2 p. m.	15			71	
Oct. 13	6 p. m.	11.5	84	83	68	
Oct. 14	8 a. m.	6		97	63	
Oct. 14	1 p. m.	4	99	97	73	
Oct. 14	6 p. m.	2.5		99	77	
Oct. 15	8 a. m.	0		99.5	58	Drew off.

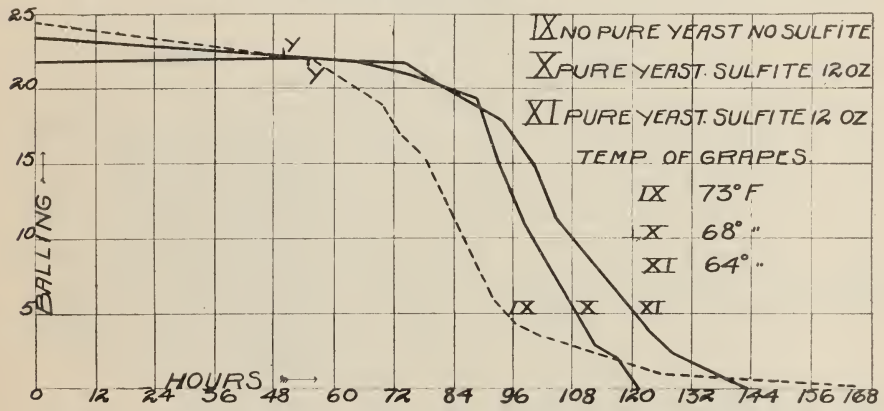


DIAGRAM 12.

Fermentation curves—Zinfandel from Contra Costa County.

The grapes used in this series were in good condition, clean and none moldy. They were thoroughly ripe and included a few raisins. The vats were filled gradually as the loads arrived, about fifty hours elapsing between the commencement and the end of filling. The sulfited vats were prevented from fermenting until the filling was complete. The unsulfited vat commenced to ferment vigorously before the last loads were crushed. The violent fermentation of the sulfited vats, therefore, took place fifteen or twenty hours later than that of the witness vat. In spite of this delay the sulfited vats became dry sooner.

In neither of the sulfited vats was there any perceptible fermentation before the addition of the starter of pure yeast. This shows the practicability of delaying fermentation of crushed grapes forty-eight hours by the use of sulfite, and their perfect subsequent fermentation by means of pure yeast. Where grapes have to be transported long distances from the vineyard to the cellar, it would undoubtedly be better to crush and sulfite them at the vineyard and transport them in this condition to the winery. The activities of molds and vinegar bacteria, so injurious to wine grapes transported in the usual way in flat cars or boxes, would thus be prevented.

Records similar to the foregoing were kept on all the red wine fermentations with similar results. All showed the prompt start and quicker finish, due to the addition of pure yeast. They showed also that sulfites in the amounts used did not delay the fermentation appreciably, and were of no value in preventing hot fermentations, though they may and, in fact, do in many cases prevent some of the worst consequences of hot fermentations by discouraging the growth of bacteria.

3. WHITE WINE FERMENTATIONS.—Observations were made on the following white wine fermentations:

Variety and origin of grapes.	Sulfite per ton.	Pure yeast.	Fermenting vessel.
I. Green Hungarian from Acampo	0	0	puncheon No. 1
II. Green Hungarian from Acampo	0	0	puncheon No. 8
III. Green Hungarian from Acampo	0	0	puncheon No. 9
IV. Green Hungarian from Acampo	12 oz.	0	puncheon No. 4
V. Green Hungarian from Acampo	12 oz.	plus	puncheon No. 2
VI. Green Hungarian from Acampo	12 oz.	plus	puncheon No. 3
VII. Green Hungarian from Acampo	12 oz.	plus	puncheon No. 6
VIII. Green Hungarian from Acampo	12 oz.	plus	puncheon No. 7
IX. Green Hungarian from Acampo	12 oz.	plus	1500-gal. cask No. 7
X. Palomino from Acampo	0	0	puncheon No. 12
XI. Palomino from Acampo	32 oz.	plus	puncheon No. 13
XII. Palomino from Acampo	32 oz.	plus	puncheon No. 14

A series of 8 puncheons was filled with the free run must of a carload of Green Hungarian grapes from Acampo. The grapes were in rather poor condition, for, being very juicy and thin skinned, many were broken, and the natural micro-organisms were present in large numbers.

Three puncheons (fermentations I, II, and III) were filled with the must and allowed to ferment spontaneously without any addition. One puncheon (IV) was sulfited at the rate of 12 ounces of metabisulfite to 200 gallons and allowed to ferment without a starter. The other four puncheons (V, VI, VII, and VIII) were sulfited in the same way, but after settling for forty-eight hours, were drawn off the sediment into clean puncheons and started with pure yeast. A larger lot of the same must was defecated in the same way in a 1,700 gallon vat and, after settling, drawn off into a 1,500 gallon cask and fermented with pure yeast.

The following partial records show the progress of some of the fermentations.

TABLE No. 49.

Green Hungarian. No sulfite. No pure yeast.

Fermentation I.			Fermentation II.*			Fermentation III.		
Hours.	Balling.	Temp.	Hours.	Balling.	Temp.	Hours.	Balling.	Temp.
0	20	66	0			0	20	
30	17	66	24	18	64.5	48	10	70
44	10	73.5	34	16		52	7.8	72.5
54	3.7	82.5	46	10	74.5	55	5.3	
68	0	79.5	50	7.5	75.0	72	0	78

*Record incomplete.

TABLE No. 50.

Green Hungarian.

Fermentation IV. Sulfite 12 ounces. No pure yeast.			Fermentation V. Sulfite 12 ounces. Pure yeast.			Fermentation IX. 1500-gallon cask. Sulfite 12 ounces. Pure yeast.		
Hours.	Balling.	Temp.	Hours.	Balling.	Temp.	Hours.	Balling.	Temp.
0	20.0		0	19.6	59.0	0	21	61.5
72	20.0		18	19.2	61.0	40	20	
90	17.0	62.5	28	18.0	63.5	136	19.7	
112	16.0	61.0	42	16.0	65.5	160	17.0	68.0
140	15.2	63.5	50	14.0	66.5	170	15.5	73.5
146	13.0	65.0	64	9.8	72.5	194	10.0	80.5
160	7.5	71.5	69	7.5	78.0	199	6.5	83.0
165	5.2	74.5	88	4.0	77.0	218	4.0	86.0
170	4.5	76.0	93	2.5	77.0	228	3.0	88.0
189	0	79.0	98	2.0	76.0	10 days.	2.5	81.0*
			112	.5	73.5	21 days.	2.0	72.5†
			117	0	73.5	22 days.	1.5	
						24 days.	1.0	
						26 days.	.5	
						31 days.	0	

*Pumped over to aerate.
†New yeast added.

TABLE No. 51.

Green Hungarian. Sulfite, 12 ounces, and pure yeast.

Fermentation VI.			Fermentation VII.			Fermentation VIII.		
Hours.	Balling.	Temp.	Hours.	Balling.	Temp.	Hours.	Balling.	Temp.
0	18.6	61.5	0	19.1	62.5	0	18.1	62.5
21	18.4	59.0	15	19.1	59.0	16	17.9	61.5
45	18.4	62.0	25	18.5	61.5	40	17.5	61.5
69	18.2	62.0	49	18.0	62.5	64	17.5	62.5
93	18.0	64.5	63	17.0	62.5	74	16.0	68.0
117	17.5	66.0	67	16.5	69.0	88	15.0	68.0
141	15.0	66.0	71	16.0	68.0	98	13.25	68.0
151	12.5	70.0	85	14.0	68.0	112	10.5	70.0
165	9.0	73.5	95	12.0	69.0	122	10.0	
175	6.2	77.0	109	10.0	68.0	136	6.0	70.0
189	3.7	74.5	119	7.5	70.0	160	3.5	73.5
199	2.0	74.5	133	5.5	70.5	184	2.0	72.5
223	0	75	157	3.0	71.5	208	1.0	70.5
			181	2.0	70.0	232	0	70.0
			205	1.0	69.0			
			215	0	71.5			

The curves of several typical examples of these fermentations are shown in diagram 13.

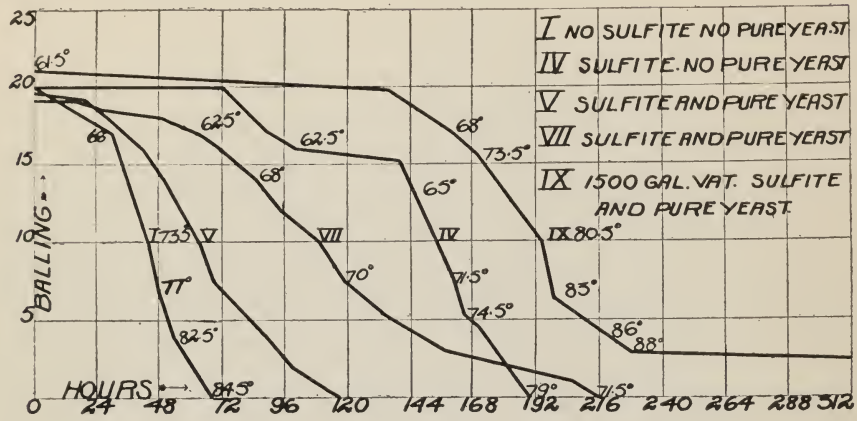


DIAGRAM 13.

Fermentation Curves of Green Hungarian.

These show a notable lengthening of the fermentation in the casks where sulfite was used. This is in strong contrast with the results of the red wine fermentations where it was found that the slight retarding influence of the SO_2 was much exceeded by the acceleration due to the use of a starter.

In No. I, in which fermentation was allowed to start spontaneously and without SO_2, the Balling per cent had fallen to 0 in less than 3 days. This showed the presence of a large amount of natural yeast due to the bad condition of the grapes. In No. IV, which received an addition of sulfite, the start of fermentation was delayed for several days. The actual tumultuous fermentation, however, was almost as rapid as that of No. I, but the total time needed before reaching 0 Balling was over 8 days. The irregularity of the curve of this fermentation is unaccounted for, but may have been due to the presence of a mixture of fermentative organisms having different degrees of resistance to SO_2 and to alcohol.

Nos. V, VII, and IX were treated with sulfite, defecated, drawn off the sediment and started with pure yeast. The regularity of Nos. V and VII is in marked contrast with No. IV and is doubtless due to the uniformity of the yeast. The retarding influence of the SO_2 is very marked in No. VII but much less so in No. V. This seems to be due to the fact that in No. VII the yeast was added 49 hours after the sulfite and in No. V 68 hours. The earlier addition of the yeast exposed it to larger amounts of free SO_2 and thus weakened its fermenting power. No ill effect resulted from this slackening of the fermentation as both

musts became perfectly dry without interruption. The slower fermentation of No. VII in fact resulted in a lower maximum temperature than in No. II, which is an advantage. Sulfurous acid thus seems to be capable of controlling the temperature to some extent in white wine fermentation, contrary to the results shown in the red wine fermentations where no appreciable effect of this kind was noted.

In No. IX the yeast was added only 30 hours after the sulfite and we have a correspondingly greater delay in the fermentation. The delay in this case did not result in a lower maximum temperature on account of the large volume of fermenting must (1500 gallons) which retarded the escape of heat. Fermentation in No. IX did not start perceptibly until over 5 days after the addition of the yeast, while in the sulfited musts in puncheons it was noticeable in from 24 to 48 hours. The fermentation after it started was rapid, owing to the conservation of the heat. It became very slow, however, by the ninth day while the saccharometer still showed 3 per cent Balling. By the tenth day the sugar had fallen only 2.5 per cent Balling. A thorough aeration on this day stimulated the fermentation slightly but on the twenty-first day 2 per cent Balling still remained; only .5 per cent Balling having been eliminated in 8 days.

It was evident that the yeast was weak so that on this day a new addition of pure yeast was made. The fermentation revived and one and a half per cent of sugar disappeared in 5 days. The wine finally reached 0 per cent Balling on the thirty-first day.

The length of this fermentation was due in the first place to the use of more sulfurous acid than was necessary for the grapes, which only had 21 per cent Balling and for the low temperature of the must which was 61.5° F. at the start. In No. V, where sufficient time was allowed for the SO_2 to combine before adding the yeast, no harm was done. In No. VII, where the must was placed in a puncheon, there was sufficient aeration to keep the fermentation going. In No. IX, however, the yeast was added too soon and the large size of the fermenting cask diminished the amount of aeration, resulting in a deficiency of oxygen for the yeast and a slower disappearance of the free SO_2.

All these cellar tests corroborate the conclusion drawn from the laboratory tests reported in Part II that the effect of sulfurous acid is due almost entirely to the free SO_2, and that the SO_2 remains free longer and in larger amounts in must alone than in the whole mass of crushed grapes. Contrary to the usual practice, therefore, more sulfurous acid is needed and should be used in red wine fermentations than in white.

In fermentation X, XI, and XII the effect of very large doses of SO_2 on excessively moldy grapes was tried. The results were not favor-

able. All the good effects of sulfurous acid were obtained with small doses, and large doses simply delay the fermentation without any corresponding advantage and very large doses injure the flavor of the wine.

In a general way, the conclusion is that for the conditions under which our tests were made, for grapes of moderate sugar contents and in cool weather about 8 ounces to 12 ounces of potassium metabisulfite per ton give the best results in the fermentation of red wine, and about half of this amount is all that is needed in the defecation and fermentation of white wine.

These comparatively small amounts are sufficient to paralyze immediately all the micro-organisms present, which is the main object, and then quickly change to the combined form so that they do not interfere with the work of the yeast which is added later. Where moderation of the rate and temperature of fermentation are desirable they should be obtained by cooling methods and not by the addition of excessively large doses of SO_2.

(d) *Quality of the Wines.*—In estimating the influence of the character of the fermentation on the quality of the wine, we must take into consideration the data supplied by tasting, chemical analysis and microscopical examination. These are all necessary to determine how well the yeast has done its work, how well the work of injurious micro-organisms has been controlled and what the probable future development of the wine will be.

1. SUGAR. Owing to the coolness of the season there was very little trouble in fermenting the wines down to dryness. What little difficulties were encountered show clearly the utility of the proper use of sulfurous acid in this respect. Wine in five of the storage casks showed appreciable amounts of unfermented sugar when analyzed on February 3, 1912.

TABLE No. 52.

Wines showing unfermented sugar.

Wines.	Sugar, per cent.			Vol. acid.
	Dec. 16.	Feb. 3.	April 10.	
I, II. Zinfandel, no sulfite, no yeast	.63	.65	--------	.140
X, XI. Zinfandel, sulfite 12 ounces, plus yeast	--------	.34	--------	.078
XV. Barbera, sulfite 5 ounces, no yeast	1.60	1.60	.7	.075
IX. Green Hungarian, sulfite 12 ounces, plus yeast	--------	.31	.3	.074
XI. Palomino, sulfite 36 ounces, plus yeast	--------	1.19	--------	.120

The Zinfandel of fermentations I and II which was fermented in the usual manner without sulfite or pure yeast showed .63 per cent of

unfermented sugar. This would not have been serious if the wine had been sound, but the volatile acid had increased to .14 per cent and a microscopical examination showed very large numbers of long rod-shaped bacteria. Such a wine as this, if left to itself, is sure to spoil completely during the first warm weather of spring.

The Zinfandel of fermentations X and XI which had been sulfited and fermented with pure yeast, showed .34 per cent of unfermented sugar. The volatile acid, however, was normal at .078 per cent and the microscope showed yeast cells of uniform appearance with only an occasional bacterial rod. Such a wine can be safely handled by the ordinary cellar methods and will ferment prefectly dry during the spring.

The Barbera, fermentation XV, which was sulfited lightly, but allowed to ferment with its own yeast showed 1.6 per cent of sugar on February 3d. The volatile acid was normal. The microscope revealed many yeast cells of various forms—ellipsoideus, pastorianus, and apiculatus types and a few bacteria. By April 10th the sugar had fallen to .7 per cent and there was every indication that the wine would ferment to dryness and remain sound.

The Green Hungarian, white fermentation IX, in 1,500 gallon cask, had been defecated with 12 ounces of metabisulfite and fermented with pure yeast. The fermentation had been very long and on February 3d the wine still showed .3 per cent of unfermented sugar, but the volatile acid was quite normal, .074 per cent. The microscope showed the sediment to be almost entirely composd of yeast cells, only a very few bacteria being found. This wine was cloudy and inferior to that fermented in puncheons but will undoubtedly finally become dry and clear and is in no danger of spoiling.

The Palomino, white fermentation XI, was made from extremely defective grapes, crushed, moldy and smelling of vinegar. The yeast was evidently injured by the large quantity of sulfite used, and the sulfited wine still showed 1.19 per cent of sugar when a puncheon of the same must fermented without sulfite was practically dry. The sediment showed an abundance of yeast and very few bacteria, so that there is no doubt that the wine will complete its fermentation in time. The comparatively high volatile acidity, .12 per cent, is probably caused by abnormal work of the yeast due to its weakened condition. This high volatile acidity does not indicate any danger of spoiling as it would if caused by bacteria, and the wine is in no danger of becoming unsound. A much smaller amount of sulfite, however, would undoubtedly have given better results without causing the "sulfur" taste which the wine still shows.

2. VOLATILE ACIDITY. In Tables 53 and 54 are given the various determinations of the volatile acidity of the wines made at intervals from a few days after the completion of fermentation until six months after.

TABLE No. 53.

Progress of volatile acidity.

Red Wines.	Volatile acid, per cent.					
	Oct. 16.	Oct. 18.	Nov. 14.	Dec. 16.	Feb. 3.	April 10.
I, II. Zinfandel; no sulfite, no yeast	.073	.105	.112	.115	.140	--------
III. Zinfandel; sulfite 5 ounces	--------	.096	.092	--------	.089	--------
IV, V. Zinfandel; sulfite 12 ounces plus yeast	.076	.086	.082	.082	.080	--------
VI. Petite Sirah; no sulfite, no yeast	--------	.088	.084	.080	.077	.114
VII, VIII. Petite Sirah; sulfite 10 ounces plus yeast	.055	.076	.076	.078	.078	.073
IX. Zinfandel; no sulfite, no yeast*	--------	--------	.061	.082	.079	.081
X, XI. Zinfandel; sulfite 12 ounces plus yeast	.064	.080	--------	.082	.078	.078
XII, XIII. Bouschet; sulfite 8 ounces plus yeast	--------	--------	--------	.074	.070	.096
XV. Barbera; sulfite 5 ounces	--------	--------	--------	.061	.059	.075

* This wine was stored in 180-gallon puncheons. All the others in 1500- to 3500-gallon casks.

TABLE No. 54.

Progress of volatile acidity.

White Wines.	Volatile acid, per cent.			
	Nov. 6.	Dec. 16.	Feb. 3.	April 10.
I. Green Hungarian; no sulfite, no yeast	.064	.066	.065	--------
III. Green Hungarian; no sulfite, no yeast	.070	.073	.071	.072
IV. Green Hungarian; sulfite 12 ounces, no yeast	.048	.070	.065	--------
V. Green Hungarian; sulfite 12 ounces plus yeast	.064	.079	.076	.076
VII. Green Hungarian; sulfite 12 ounces plus yeast	.065	.066	.075	.065
VIII. Green Hungarian; sulfite 12 ounces plus yeast	.061	.064	.068	.066
IX. Green Hungarian; sulfite 12 ounces plus yeast*	.061	.066	.067	.074
X. Palomino; no sulfite, no yeast	--------	.068	.065	.065
XI. Palomino; sulfite 36 ounces plus yeast	--------	.100	.100	.120
XII. Palomino; sulfite 36 ounces plus yeast	--------	.080	.090	.102

*This wine was stored in a 1500-gallon cask. All the others in 180-gallon puncheons.

As shown by Table 53 the volatile acidity in all the sulfited red wines was normal, showing a clean fermentation and absence of bacterial action. In one wine, Zinfandel I and II, fermented in the ordinary way without sulfites, the volatile acidity commenced to rise almost immediately after the end of the tumultuous fermentation and continued so long as the observations extended. This is a case of a "stuck" wine and shows the ordinary course of a young wine containing a remnant of unfermented sugar and large quantities of bacteria.

In the Petite Sirah No. VI, also an ordinary fermentation, the volatile acidity remained normal for about four months following the vintage. During the first warm weather of spring, however, the volatile acid commenced to rise and by April 10th it was 60 per cent higher than that of Petite Sirah VII and VIII fermented with sulfite and pure yeast. In this case the ordinary fermentation and that in which sulfite was used were successfully brought to 0 Balling and the wines were both practically dry when analyzed. The sulfited wine showed, under the microscope, a clean sediment with very few bacteria, while the unsulfited showed large numbers of bacteria almost from the beginning. In April these bacteria were very numerous. They consisted of long and short rods and were not vinegar bacteria. This experiment shows that even the completion of the fermentation to apparent dryness does not always prevent the increase of volatile acid in unsulfited wines.

The remaining unsulfited wine, Zinfandel No. IX shows a perfectly normal volatile acidity up to April 10th, being in this respect practically identical with the sulfited Zinfandel Nos. X, XI made from the same grapes. When analyzed on February 3d it showed only .21 per cent of unfermented sugar while the sulfited wine showed .34 per cent at the same date. A microscopical examination of the sediment showed very few bacteria in the sulfited wine but a larger number in the unsulfited.

Both wines were prefectly sound and if handled properly were in no danger of deteriorating. That this wine No. IX kept better than the others fermented in the same way without sulfiting seems to be due to the fact that it was stored in puncheons immediately after fermentation. This view is corroborated by the fact that there was no abnormal increase of the volatile acid in any of the Green Hungarian wines stored in puncheons whether sulfite was used or not. The increase of volatile acidity after fermentation is due to anaerobic bacteria which grow the more vigorously the better they are protected from oxygen. In a puncheon, the aeration through the pores of the wood is much greater than in a large vat. In a puncheon of 180 gallons the ratio of the surface to the volume is about $4\frac{1}{2}$ times greater than in a cask of 1500 gallons. Supposing the wood of both casks to be of the same kind and thickness, therefore, the wine in a puncheon is aerated by the slow penetration of air through the staves and head $4\frac{1}{2}$ times as much as the wine in a 1500 gallon cask. This is sufficient to account for the quicker finish of the fermentation of wines stored in small casks, the smaller growth of anaerobic bacteria and the better development of the wine. The use of sulfurous acid prevents the excessive growth of bacteria even in the large casks, but it does not overcome the slowness of the after fermentation or of the development of the wine.

3. FIXED ACIDITY. The influence of sulfite and pure yeast on the fixed acidity is masked in the red wines by unavoidable variations in the grapes and by the difficulty of obtaining a representative sample before fermentation. The errors thus introduced are likely to neutralize each other to some extent in the means of all the wines. The figures representing the average acidity found in the red grapes and in the corresponding red wines may safely be taken as representing the general direction of the influence in this respect. In the white wines the observational errors are much smaller. The white grapes were all crushed into the same vat and the must from this vat distributed into the various vats and puncheons in which the experiments were made. The variations in the composition of the raw material and in the samples taken for analysis are much less in this case.

TABLE No. 55.

Influence of sulfurous acid on fixed acidity.

	Alcohol.	Fixed acid.			
		No sulfite.		Sulfite.	
		Must.	Wine.	Must.	Wine.
RED WINES.					
Zinfandel, I, II	11.85	1.06	.51		
Zinfandel, III; 5 ounces sulfite	12.60			1.00	.38
Zinfandel, IV, V; 12 ounces sulfite	12.30			.97	.63
Petite Sirah, VI	11.65	.81	.40		
Petite Sirah, VII, VIII; 10 ounces	11.90			.84	.36
Zinfandel, IX	13.40	.95	.53		
Zinfandel, X, XI, 12 ounces	12.15			.96	.82
Average fixed acidity of red wines		.94	.48	.94	.55
WHITE WINES.					
Green Hungarian, I	11.30	.64	.45		
Green Hungarian, III	11.30	.64	.43		
Green Hungarian, IV; 12 ounces sulfite	11.25			.64	.57
Green Hungarian, V; 12 ounces sulfite	11.20			.64	.60
Green Hungarian, VII; 12 ounces sulfite	11.30			.64	.60
Green Hungarian, VIII; 12 ounces sulfite	11.30			.64	.55
Green Hungarian, IX; 12 ounces sulfite	11.20			.64	.51
Average fixed acidity of white wines		.64	.44	.64	.57

This table indicates that in the red wine fermentations where no sulfite was used, there was a loss of 49 per cent of the fixed acidity. This loss was reduced to 41 per cent where sulfites were used. In the white wine only 31 per cent of the fixed acidity was lost even in the ordinary fermentations, and this loss was reduced by the use of sulfites to 11 per cent.

The greater loss of fixed acidity in the red wines is accounted for principally by the more advanced stage of maturity of the grapes from which they were made. The less ripe the grapes the more of their

acidity exists in the form of free tartaric acid. This acid is soluble in all proportions in the wine and, therefore, is found *in toto* in the wine unless destroyed by acid-consuming organisms. In the riper grapes, a greater part of the acidity exists in the form of bitartrate of potash which is less soluble in the wine than in the must. A certain proportion of this acidity is therefore lost from the wine by the precipitation of cream of tartar. This loss is further increased by the production of alcohol in which the bitartrate is insoluble. The more alcoholic the wine, therefore, the less bitartrate can remain in solution.

The increase of acidity with the use of sulfurous acid is due, therefore, principally to its conservation of the free acid.

4. ALCOHOL. As shown by Table 56 the sulfited red wines showed a better utilization of the sugar. The ratio of the alcohol by volume in the wine to the original Balling ° of the must being on the average .55 for the sulfited wines and .52 for the parallel wines fermented without sulfites.

TABLE No. 56.

Influence of SO_2 and pure yeast on alcohol production in red wines.

Fermentation.	No sulfites.			Sulfites.		
	Balling ° of must.	Alcohol in wine.	Ratio.	Balling ° of must.	Alcohol in wine.	Ratio.
Zinfandel, I, II	24.8	11.85	.48			
Zinfandel, III				22.3	12.60	.57
Zinfandel, IV, V				22.5	12.40	.55
Petite Sirah, VI	22.5	11.85	.53			
Petite Sirah, VII, VIII				22.1	11.85	.54
Zinfandel, IX	24.5	13.40	.55			
Means	23.8	12.37	.52	22.3	12.28	.55
Theoretical ratio	23.8	13.57	.57	22.3	12.49	.56

The ratio of alcohol to Balling ° should be higher with the grapes containing more sugar, as the proportion of non-sugar extract included in the Balling ° is less. In the last line of the table is given the amount of alcohol and the ratio which should have been obtained according to Salleron's tables. This shows an average loss of alcohol in the unsulfited wines of 1.2 per cent and in the sulfited of only .21 per cent. These losses represent partly, unfermented sugar, and partly, sugar and alcohol wasted by wild yeasts, bacteria and high temperatures.

5. COLOR OF RED WINES. It is difficult to determine exactly the influence exerted by the SO_2 on the color on account of unavoidable variations in the grapes of the various vats. On the whole, there is no doubt, however, that it was favorable. The wines from the sulfited vats in nearly all instances were of better tint and more intense colors than those from the corresponding unsulfited vats.

TABLE No. 57.

Influence of SO_2 and pure yeast on the color of red wines.

Fermentation.	No sulfite.		Sulfite.	
Zinfandel, I, II	5 VR	44		
Zinfandel, III			4 VR	35
Zinfandel, IV, V			4 VR	52
Petite Sirah, VI	2 VR	93		
Petite Sirah, VII, VIII			1 VR	121
Zinfandel, IX	3 VR	35		
Zinfandel, X, XI			3 VR	66
Means		56		69

In the Zinfandel of the first series the sulfited wines show a slight improvement of tint but the intensity of the color is in one case slightly less and in the other slightly more than in the unsulfited. This is really a favorable showing, as the unsulfited grapes were riper and therefore more deeply colored. In the other series the sulfited showed from 30 per cent to 80 per cent more color than the corresponding unsulfited wines. The average of all the wines showed an increase of about 23 per cent.

6. CONDITION. The condition of a young wine means its degree of limpidity or the amount and character of suspended solid matter it contains. A wine during and immediately after fermentation is said to be "murky or muddy." After the main part of the yeast and other sediment has settled it is said to be "cloudy." When it is sufficiently freed from floating matter to be transparent it is called "clear." The final stage of limpidity is reached when the most careful scrutiny fails to detect any visible floating particles. The wine is then said to be "bright."

These various stages occupy various periods according to the soundness of the wine and the methods of handling. A wine will lose its gross sediment and reach the "cloudy" stage within a few days after the end of perceptible fermentation. The length of time during which wines remain "cloudy" will vary from a few days to many months. A perfectly sound and properly fermented wine should be "clear" within three weeks of pressing in the case of red wine and within six or eight weeks in the case of white. Clearing is delayed by unfermented sugar and the presence of many bacteria. It is hastened and facilitated by suitable aeration and temperature.

The rapid clearing of the wine was a constant effect of the use of sulfurous acid and pure yeast owing to the more perfect elimination of the sugar and the prevention of bacterial and wild yeast action.

7. SULFUROUS ACID REMAINING IN THE WINE. In Table 58 is shown the amount of total SO_2 found in the wines fermented in the presence of sulfites. For the white wines the amount of free SO_2 found is also given. The determinations were made at the beginning of February when the wines were about four months old.

TABLE No. 58.

Sulfurous acid remaining in the wine.

Red wines.	Sulfite added per ton.	Equivalent SO_2 per cent.	Per cent of SO_2 found in wine.
Zinfandel, III	5 oz.	.0078	.0040
Zinfandel, IV, V	12 oz.	.0188	.0076
Petite Sirah, VII, VIII	10 oz.	.0156	.0076
Zinfandel, XXI	12 oz.	.0188	.0096
Alicante Bouschet, XII, XIII	8 oz.	.0125	.0108
Zinfandel, XVI	8 oz.	.0125	.0108
Miscellaneous	8 oz.	.0125	.0096
White wines.	**Free SO_2 per cent found in wine.**	**SO_2 per cent added.**	**Per cent of SO_2 found in wine.**
Green Hungarian, IV	.0013	.0188	.0154
Green Hungarian, V	.0013	.0188	.0149
Green Hungarian, VII	.0012	.0188	.0154
Green Hungarian, VIII	.0010	.0188	.0159
Green Hungarian, IX	.0013	.0188	.0151
Palomino, XI	.0015	.0500	.0418
Palomino, XII	.0015	.0500	.0340

These analyses show that at four months from 14 per cent to 60 per cent of the total sulfurous acid added to the must had disappeared from the red wine and from 16 per cent to 30 per cent from the white. There is, therefore, no danger of exceeding the legal limit, which is sufficiently high for all the legitimate purposes of the wine-maker. Even in the Palomino, where from 3 to 4 times as much sulfite was used as was necessary or desirable, the SO_2 in one puncheon had already fallen below the legal limit.

The amount of free SO_2 was very low in all the wines—so low that in the red wines its exact determination was difficult.

TABLE No. 59.

Composition of experiment wines. Analyses made February 3, 1912.

Fermentation.	Must.		Wine.										
	Balling per cent.	Acidity.	Extract.	Sugar.	Ash.	Total Acidity.	Volatile Acidity.	Alcohol.	Tannin.	Color.	Sulfates.	Glycerine.	SO_2.
Zinfandel, I, II	24.8	1.06	3.63	.65	.304	.65	.140	11.85	.220	5 VR 44	.0343		
Zinfandel, III	22.3	1.00	3.03	.15	.350	.47	.089	12.60	.225	4 VR 35	.0720		.0040
Zinfandel, IV, V	22.5	.97	3.30	.16	.347	.71	.080	12.40	.259	4 VR 52	.0590	.9430	.0076
Petite Sirah, VI	22.5	.81	2.80	.11	.303	47	.077	11.85	.389	2 VR 93	.0220	.7380	
Petite Sirah, VII, VIII	22.1	.84	3.35	.14	.399	41	.078	11.85	.432	1 VR 121	.0590	.9760	.0076
Zinfandel, IX	24.5	.95	2.89	.21	.207	61	.079	13.40	.170	3 VR 35	.041		
Zinfandel, X, XI	22.3	.96	3.56	.35	.220	90	.078	12.25		3 VR 66	.051		.0096
Alicante Bouschet, XII, XIII	22.4		3.80	.26	.354	.52	.070	12.05	.254	2 VR 42	.056		.0108
Barbera, XV			5.14	1.61	.120	1.00	.059	11.80	.273	2 VR 73	.043		
Zinfandel, XVI			3.62	.19	.558	.53	.056	11.60	.254	2 VR 65	.063		.0085
Misc., XVII, XVIII, XIX, XX			3.36	.28	.335	.62	.077	12.00	.461	2 VR 121	.061		.0096
Green Hungarian, I	20.0	.64	2.01	.12	.238	.45	.065	11.30			.048	.5667	
Green Hungarian, III	20.0	.64	2.20	.21	.286	.42	.071	11.30			.053	.6771	
Green Hungarian, IV	20.0	.64	2.10	.08	.352	57	.065	11.25			.030	.5971	.0154
Green Hungarian, V	19.6	.64	2.30	.12	.205	58	.075	11.30			.050		.0149
Green Hungarian, VII	19.1	.64	2.10		.242	.60	.075	11.3			.050	.4736	.0153
Green Hungarian, VIII		.64	2.20	.19	.281	.60	.061	11.3			.056	.4107	.0159
Green Hungarian, IX	21.0	.64	2.30	.29	.194	.50	.067	11.2			.043		.0151
Palomino, X	24.5		2.56	.16	.272	.38	.065	14.0			.051	.7590	
Palomino, XI	25.0		4.37	1.19	.380	52	.100	13.5			.067	.7033	.0418
Palomino, XII	25.0		3.04	.21	.392	.46	.090	13.7			.078	.8917	.0340

V. SUMMARY AND CONCLUSIONS.

I. Introduction.

The principal modern improvements in wine-making are the intelligent use of sulfurous acid, pure yeast and temperature control.

II. Use of Sulfurous Acid in Wine-Making.

The use of sulfurous acid in wine-making is of great antiquity but has been much developed and perfected within the last twenty-five years.

Approved by all enological experts.

Present legal limitations satisfactory but unnecessary for wine-making.

Fumes of burning sulfur the cheapest source of SO_2 and the best for disinfection purposes.

For the control of fermentation this source is uncertain and difficult to regulate.

From 1.5 pounds to 2 pounds of sulfur can be burned in a 1000 gallon cask.

This will supply about .02 per cent of SO_2 to the must as a maximum.

In practice usually much less than this is absorbed.

Liquid SO_2 appears to be the best form for the control of fermentation but is at present unavaliable in California.

Solutions of SO_2 in water and alcohol are unreliable.

Potassium metabisulfite is the best form at present available.

SO_2 disappears less rapidly from must than from water.

SO_2 combines rapidly with certain substances in the must into which it is introduced.

The combination of SO_2 is more rapid in must from dried grapes than in that from fresh.

About one half of the SO_2 disappears during the fermentation of red wine.

SO_2 is more stable in wine than in must.

The increase of sulfates in the wine due to sulfiting is very small.

Very ripe grapes require more SO_2 than those which are less ripe both because they are more liable to bacterial attack and because they neutralize more of the antiseptic effect of the SO_2.

This neutralization of the antiseptic effect seems to be due to neither excess of sugar nor to deficiency of acidity.

The more solid matter present the less effective the SO_2. Therefore, cloudy must requires more than clear and the crushed grapes in red wine fermentations require about twice as much as the must in white wine fermentations.

The neutralizing effect of must is increased by heating to 120° C. under pressure.

The neutralization of the antiseptic power of the SO_2 is due to its combination with components of the must.

Free SO_2 has more than thirty times as much disinfecting power as combined SO_2.

Exposure to 300 milligrams per liter of free SO_2 will kill wine yeast in 24 hours.

About 50 milligrams per liter of free SO_2 will prevent fermentation with wine yeast.

Free SO_2 is about sixty times as effective in preventing fermentation as combined SO_2.

Very small amounts of SO_2 (5 ounces of potassium metabisulfite per ton in most cases) are sufficient to prevent all growths of molds and wild yeasts and to insure a pure fermentation when a starter of wine yeast is used.

100 milligrams of SO_2 per liter (6 ounces of potassium metabisulfite per ton) eliminate over 99.9 per cent of the active cells of microorganisms from the must.

Wine yeast does not become inured to SO_2 but, on the contrary, exposure increases its sensibility. The reputed training of yeast to withstand SO_2 is a fallacy.

Wine yeast is less sensitive to SO_2 than any of the common yeasts, molds or bacteria occurring in grapes and wine.

By properly timing the sulfiting and the addition of the starter the full effect of the maximum amount of free SO_2 is exerted on the injurious organisms and the yeast is exposed only to the minimum amount of free SO_2.

III. Utility and Methods of Application of Pure Yeast in Wine-Making.

Favorable results from the use of pure yeast in wine-making were obtained by the station nineteen years ago.

Pure yeast has been used regularly with success for many years in several Californian wineries.

The necessity of a proper selection of yeast has been demonstrated.

Champagne and Burgundy yeasts have been found especially suitable to Californian conditions.

Grapes from all regions investigated, whatever their variety, condition or stage of ripeness showed large numbers of mold spores and wild yeasts. The wine yeast was never present in large numbers and was usually outnumbered many thousand times by the injurious microorganisms.

Solid cultures of pure yeast were found more stable and reliable than liquid cultures.

Direct application of the pure yeast received from a laboratory

involves the buying and transporting of large amounts and is too expensive.

The previous preparation of a starter from a small culture received from a yeast laboratory is preferable.

With a little practice and care any intelligent wine-maker can prepare a starter.

A starter should be used when it has its maximum efficiency which is about the stage at which the Balling ° has been reduced one half. The efficiency does not diminish much until all the sugar has disappeared.

Expensive and complicated yeast propagators are unnecessary in wineries.

A simple and cheap apparatus can be devised suitable for wineries of any size and requiring the labor of only one man in the largest winery.

IV. Tests of the Use of SO_2 and Pure Yeast In a Winery.

The use of SO_2 and of pure yeast witout the aid of cooling appliances was tested in a small winery.

The growth of molds and wild yeasts was completely prevented by the use of SO_2.

Large quantities of SO_2 in white wine making, even with grapes in very bad condition, were found unnecessary and inadvisable.

The yeast apparatus used was satisfactory and insured practically pure fermentations.

SO_2 and pure yeast can be used to insure the completion of fermentation and to prevent "stuck" wines even without cooling devices if the wine is handled in small casks.

In large casks and vats where the end fermentation is often slow the use of SO_2 much decreased the danger of bacterial deterioration.

The volatile acidity is uniformly lower in the sulfited wines.

The fixed acidity is protected by the use of SO_2 and the sulfited wines show a higher total acidity than the others.

The use of sulfites gave an increase in the alcohol of the wines of about 1 per cent.

The color of the sulfited red wines was improved in both tint and intensity.

The treated wines cleared more rapidly and showed sounder sediments.

The amount of SO_2 remaining in the wines was much below the legal limitation except where unnecessarily large amounts were used.

STATION PUBLICATIONS AVAILABLE FOR DISTRIBUTION.

REPORTS.

1896. Report of the Viticultural Work during the seasons 1887–93, with data regarding the Vintages of 1894–95.

1897. Resistant Vines, their Selection, Adoption, and Grafting. Appendix to Viticultural Report for 1896.

1902. Report of the Agricultural Experiment Station for 1898–1901.

1903. Report of the Agricultural Experiment Station for 1901–03.

1904. Twenty-second Report of the Agricultural Experiment Station for 1903–04.

BULLETINS.

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No. 128. Nature, Value, and Utilization of Alkali Lands, and Tolerance of Alkali. (Revised and Reprint, 1905.)

133. Tolerance of Alkali by Various Cultures.

147. Culture Work of the Sub-stations.

162. Commercial Fertilizers. (Dec. 1, 1904.)

164. Poultry Feeding and Proprietary Foods.

165. Asparagus and Asparagus Rust in California.

167. Manufacture of Dry Wines in Hot Countries.

168. Observations on Some Vine Diseases in Sonoma County.

169. Tolerance of the Sugar Beet for Alkali.

170. Studies in Grasshopper Control.

171. Commercial Fertilizers. (June 30, 1905.)

174. A New Wine-cooling Machine.

176. Sugar Beets in the San Joaquin Valley.

177. A New Method of Making Dry Red Wine.

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32. White Fly Eradication.
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46. Suggestions for Garden Work in California Schools.
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52. Information for Students Concerning the College of Agriculture.

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62. The School Garden in the Course of Study.
63. How to Make an Observation Bee Hive.
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67. Development of Secondary School Agriculture in California.
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69. The Extermination of Morning-Glory.
70. Observations on the Status of Corn-growing in California.
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